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VOL. I, No. 4 APRIL 1949

# PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

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**International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases.**

**Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol.**

**Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten**

*Issued under the auspices of the Netherlands Society of Agricultural Science.*



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Printed in the Netherlands

Plant and Soil 1, 277-371. The Hague, April 1949.

Plant and Soil I, no 4 April 1949

# THE AVAILABILITY AND FIXATION OF COPPER IN SWEDISH SOILS

by KARL LUNDBLAD, OLOF SVANBERG and PER EKMAN

The Royal Agricultural College, Uppsala, and the State Agricultural Experiment Institute of Sweden

I. Introduction.
II. Methods.
III. Analyses and experiments on peat soils.
IV. Analyses and experiments on mineral soils.
V. Further comparisons between the effects on the yield, of fertilizing with copper sulphate on peat soils and on mineral soils with different contents of organic matter.
VI. Lime and pH in their relation to the availability of copper to plants.
VII. Summary and discussion.

I. *Introduction.* For a number of years, deficiency diseases in grain crops ("white tip" disease *) an abnormally large proportion of light grain, and reduced grain yields) have been observed on some Swedish soils, chiefly peat soils. This matter was made a subject of investigation in fertilizing experiments with copper sulphate and other salts of minor and secondary elements by Osvald (1930), Lundblad (1939) and Undenäs (1937). It was noted that fertilizing with sulphate of copper as a top dressing could bring about great increases in the yield of grain crops and a much more uniform and healthy vegetation both of grain crops and of fodder grasses, than was recorded on the control plots.

The results of this work, however, were frequently rather variable from year to year on the same soils. The tendency towards white-tip disease and reduced yields among the controls was in no way a

*) The disease is sometimes called "yellow tip" — a translation of the Danish name "gulspidssyge" (Mortensen, Rostrup and Kølpin Ravn 1911) — or "reclamation disease" — a translation of the Dutch name "ontginningsziekte" (Elema 1920).

regularly occurring event. For this reason it seemed necessary to combine a number of new copper fertilizing experiments in the field with analyses of copper in the soil and in the vegetation and in this way to arrange longterm experiments in order to study the behaviour of the copper and the variability of the content of this metal in the plants on fertilized and control plots from year to year.

Analyses of the copper content in Swedish hay samples have been made earlier by Svanberg and Nydahl (1941). In the present investigation a more detailed survey has been made concerning both soils and hay samples from all over the country in order to obtain a better idea of the copper status in different parts of Sweden and to find out those soils, on which a low grain production may be caused by a shortage of this biogenic metal. This work shows that rather large areas of mineral soils (mostly sandy soils) in the south of Sweden, besides peat soils in several parts of the country, are rather deficient in copper.

Some of the results of these investigations, mainly carried out over a period of about eight years,would appear to be of international scientific interest. For this reason they will be briefly summarized in this communication. In the authors opinion, their importance chiefly concerns questions as to the availability and fixation of the copper in different types of Swedish soils.

At the same time, attention may be drawn to the condition of farm animals, chiefly dairy cattle, in their dependence upon the copper content of the hay, pasture and other fodder stuffs. In this connection the following observations may be mentioned. A copper content of the rations amounting to about 4 ppm on a dry matter basis, seems capable of preventing disturbances in the livestock, caused by a shortage of copper. These disturbances refer mainly to troubles in young animals, but occasionally a pronounced lack of appetite (or an appetite for things unsuitable for fodder) may also occur in cows. Sometimes great difficulties arise in the breeding of young animals combined with sterility and the need for a great number of services of cows in order to make them pregnant. These difficulties disappear after the use, for some time, of copper sulphate in the manner, mentioned as follows. In most parts of the contry, where the danger of reduced grain yields and white tip disease is not prevalent, the Cu figures in hay and pasture are about 6–7 ppm, whilst on deficient soils the Cu content may sink to 1–2 ppm or even lower values. In the northern part of the country the greatest economical losses, attributable to deficiency of copper in the soils, occur in animal husbandry, owing to the predominance of this form of production and the relatively unimportant areas of grain crops. In the southern

parts of Sweden, on the other hand, greater losses occur in cereal crops at the same time the cattle, may suffer to a less significant degree. In these parts of Sweden a larger percentage of clover in the pastures and the leys secures a higher copper content in the fodder crops than is the case in the North, where long-term grassland (with grasses only) predominates. The use of molasses and imported concentrates (oil cake and meals), which are rather rich in copper, is able to prevent losses in animal production due to a deficiency in this metal. During the war, however, when the supply of these fodder stuffs was very restricted, fodder phosphates such as sodium or calcium phosphate with an addition of about 0.5 pct commercial copper sulphate were used more and more. At the present time such mixtures are very popular in large parts of Sweden.

II. *Methods.* Among the methods used in this work the following may be mentioned.

The fertilizing experiments in the field generally consist of 4–6 different treatments, each treatment having 4–5 replications. The single plots are, as a rule, 20–50 $m^2$ each. In some of the experiments copper sulphate as a top dressing was applied as dry, finely ground substance, in others in water solution. After the second year of observations, differences between the effects of different methods of application were small and insignificant.

The determinations of copper in samples of vegetation and soil were carried out in the following way, according to improvements in the technique made by E k m a n.

For the digestion of a number of samples at the same time, a digestion apparatus was constructed, each Kjeldahl flask being provided with a gas burner and glass bulb, as shown in the figure (Fig. 1).

The acid fumes are sucked away with water pumps and absorbed in water, which may be renewed when necessary. The small side tube on the conical tube of the apparatus permits the addition, if necessary, of further reagents to the digestion flasks without their removal from the equipment.

1.25 g of the air dried sample is weighed into a 50 or 100 ml Kjeldahl flask. 10 ml conc. nitric acid and 3 ml 70% perchloric acid are added. If the mixture foams violently, the reaction is retarded by dipping the flask into cold water. Anti-foaming alcohols or similar additions must not be used in contact with perchloric acid because of the danger of explosion.

For samples, poor in copper, more of the substance must be taken for analysis and the quantities of the reagents must be increased proportionally. For samples of mineral soils or organic soils with a relatively high mineral content a quantity of the nitric acid somewhat smaller than that mentioned above is used.

The sample is left under the hood for about 1 hour. After this it is slowly heated to boiling, when the nitric acid oxidizes the main portion of the organic matter. After boiling away the excess nitric acid, the temperature is raised and the perchloric acid now oxidizes the remaining organic matter (Caution: Eye-protecting glasses: see note). When the sample is nearly

colourless it is boiled for 5 minutes more in such a manner that the perchloric acid condenses in the neck of the flask. After cooling, 15 ml of 0.5 M. ammonium citrate is added, one drop of 1% phenolphtalein soln. and 6 M. ammonia (about 6 ml) until distinctly red in colour. The sample is heated half an hour on a steambath in order to dissolve salts which may have precipitated.

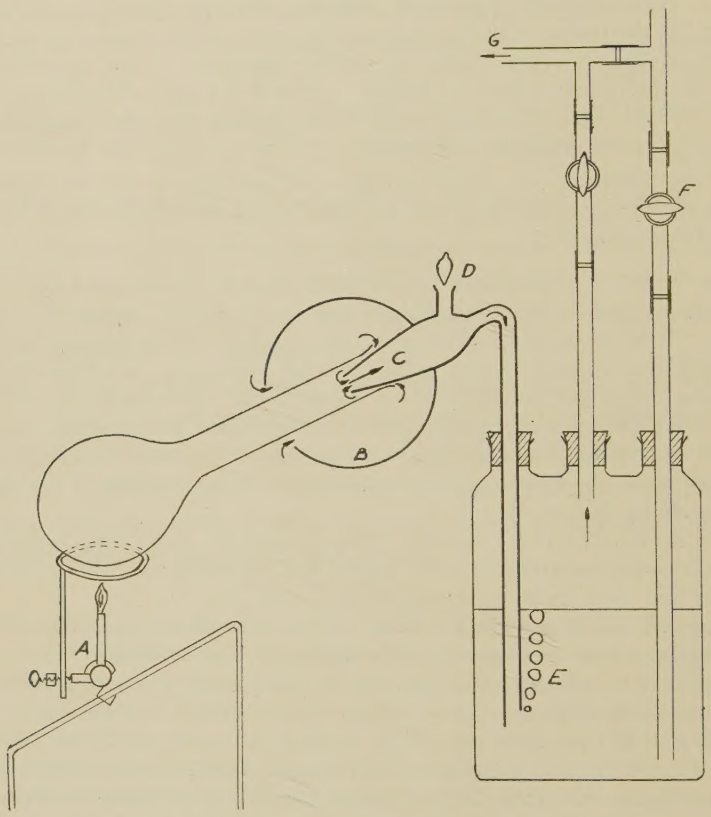


Fig. 1. Apparatus for wet combustion with nitric and perchloric acid.

*A*. Burner and support are adjustable according to size of flasks.
*B*. Glass bulb, serving as a receiver for drops and "gas buffer".
*C*. The conical tube extends a few cm inside the neck of the flask, and prevents acid fumes from escaping and condensing on the outside of the neck.
*D*. Delivery tube for extra reagent during the course of digestion.
*E*. Water, in which the hot acid fumes are cooled and dissolved.
*F*. Glass tube for emptying and filling the Woulff bottle with water.
*G*. To suction; an ordinary filter pump will take care of four parallel digestions.

After cooling the sample, 10 ml 0.1% sodium-diethyl-di-thiocarbamate in water (i.e. 10 mg reagent, which is sufficient for about 1 mg of copper) is added. The solution is poured off into a 100 ml separatory funnel, containing 10 ml carbon tetrachloride. The flask is washed three times with a few ml of ammonium citrate solution, care being taken to leave coarse particles of sand etc. in the Kjeldahl flask. The separatory funnel is shaken vigorously for two minutes. The liquids having separated, the lower layer is drawn off into another separatory funnel, where it is shaken for one minute with 25 ml. 0.5-M. Cu-free ammonia in order to remove discolouring impurities like iron etc. The solution in $CCl_4$ is filtered through a rapid paper filter (Munktells OR 55 mm or a similar brand) into a glass stoppered bottle, which is kept in the dark until the analysis is carried out.

The copper content is determined by measuring the extinction in light at wave length 440 m$\mu$, where the gold coloured Cu-diethyl-dithiocarbamate has its maximum absorption (e.g. by a Beckman spectrophotometer).

The added citrate buffer, by forming soluble complexes, prevents the precipitation of phosphates and hydroxides of copper, calcium, aluminium, iron and other metals and also prevents the adsorption of copper on solid substances.

It is, of course, necessary to carry out controls with all reagents, as they are seldom completely free from copper.

The copper-diethyl-dithiocarbamate has a much greater solubility in $CCl_4$ than in water. For this reason a single extraction with $CCl_4$ is sufficient in routine analyses if the proportion $H_2O$ : $CCl_4$ is not too high. The sodium salt (= the colourless copper reagent) on the other hand is more soluble in water. After the first separation therefore, the $CCl_4$ contains only a small but undetermined excess of the reagent. One cannot be certain whether this excess is large enough to combine with all the copper which may be present as an impurity in the diluted ammonia during the second shaking. This $NH_3$ solution therefore, must either be treated with a sufficient quantity of the reagent or, preferably be completely free from copper. Otherwise the controls as well as the analytical figures may not be quite reproducible.

When samples of plants or of a purely organic soil are treated in the way mentioned above, their total content of copper is brought into solution. Copper which is fixed in the crystal lattice of primary silicaceous minerals, however, is not extracted if the time of digestion is restricted. For samples of a mineral soil the analytical figure obtained gives a value for the "acid-soluble" or — approximately — the total exchangeable and organically combined quantity of copper, which takes part in the maintainance of the equilibrium in the soil solution.

According to Smith Brun (1946) the major portion — more than 80% of the total copper content of organic soils are extractable with suitable complex forming agents, e.g. potassium cyanide or quinaldinic acid.

*Note*: Fats and other substances with long carbon chains and some organic acids are only slightly attacked by nitric acid. When the acid is boiled away, therefore, there may remain a relatively large quantity of

organic matter, giving rise to an explosive mixture with the perchloric acid. For this reason such samples as seeds, rich in oil, animal products and molasses, etc. must first be digested with a mixture of nitric acid and sulphuric acid. Perchloric acid may then be cautiously added, dropwise, in order to complete the oxidation. It is further to be observed that neutralization of sulphuric acid requires three times as much alkali as does the same volume of perchloric acid.

In some cases it is necessary to eliminate interference due to Co and Ni, and this may be done by the addition of a few mg dimethylglyoxime before neutralization (Butler and Allen, 1942 and H. Cheftel *et al.* 1947). With regard to the determination of copper see also F. Nydahl (1939), Clare (1945), Reith (1947) and Sandell (1944).

Table I gives some values for mineral soils with respect to the total copper content after treatment with hydrofluoric acid, the copper brought into solution according to the method, described above, and the copper recovered from quantities added to the samples.

TABLE I

Examples of analytical results for copper in soils with and without addition of copper

| Soil | Loss on ignition pct | Cu added $\gamma$/g | Cu, total after treatment with HF $\gamma$/g | Cu, after digestion with $HClO_4$ $\gamma$/g | Cu, recovered of Cu added |
|---|---|---|---|---|---|
| Light clay | 6.6 | 0 | 15.1 | 7.5 | — |
| (Lanna I) | 6.6 | 8 | — | 15.1 | 7.6 |
| Light clay | 6.6 | 0 | 15.6 | 5.9 | — |
| (Lanna II) | 6.6 | 8 | — | 13.8 | 8.9 |
| | 6.6 | 16 | — | 21.6 | 15.7 |
| Sand | 5.0 | 0 | 14.7 | 7.6 | — |
| (Blombacka I) | 5.0 | 8 | — | 16.9 | 9.3 |
| Sand | 3.9 | 0 | 10.6 | 5.5 | — |
| (Blombacka II) | 3.9 | 8 | — | 13.8 | 8.3 |
| None | — | 8 | — | 7.7 | 7.7 |
| None | — | 8 | — | 7.6 | 7.6 |
| None | — | 16 | — | 15.9 | 15.9 |
| Sum | | 80 | | | 81.0 |

Treatment of a sample with hydrofluoric acid gives the total amount of copper in the soil because the silicaceous minerals are dissolved and their silica removed.

III. *Analyses and experiments on peat soils.* North of the 61° parallel, between the 61° and the 68°, especially in the interior of

Sweden, the copper content in the grassland vegetation on cultivated peat soils is without exception very markedly reduced below normal values. At the same time, the peat itself has a very low content of copper. South of the 61° parallel, i.e. between 55° and 61°, the same conditions are to be met with, but not at all without exceptions. There are, as a matter of fact, many examples of peat soils in these parts of the country, where the copper content of the peat soils is much higher, sometimes very high, and the vegetation of a quite normal composition as regards copper. In North Sweden therefore peat soils are generally poor in copper, whilst in the middle and southern parts of the country large individual variations are prevalent, as is shown by differences between the lowest and highest analytical values.

Normal values for the copper content in the vegetation in Sweden are the same as those found in the international literature on this subject. Thus, the authors found the following mean values from soils satisfactory with regard to copper content: for hay of mixed grasses 6.0 ppm, for timothy 5.8 ppm, for red clover and Alsike clover 9.8 ppm and for alfalfa 8.7 ppm, all figures on a dry matter basis. Under copper-deficient conditions the copper content of the grasses is reduced to much lower values than normal, the extremes being as low as about 0.5 ppm. The mean copper content of the grasses indicates rather well the temporary copper status of the soil.

As mentioned above, the determination of copper in soils was carried out in the same way as in the samples of vegetation. In this method for preparing the extracts, all the copper combined with organic matter will pass into solution. The same is true for all copper, which is not fixed in the crystal lattice of the mineral constituents.

Table II gives an abbreviated account of the copper content of Swedish samples of hay from peat soils in different parts of the country, whilst Table III demonstrates the values from soil analyses.

TABLE II

Copper content in Swedish samples of hay from peat soils, ppm dry matter, in different parts of the country

| Region | Number of samples | Copper, ppm | |
|---|---|---|---|
| | | Mean | Variations |
| 61°–67° "Norrland" | 188 | 2.9 | 0.5– 9 |
| 59°–61° "Svealand" | 35 | 5.0 | 1.5– 9 |
| 55°–59° "Götaland" | 31 | 6.0 | 1.5–13 |

TABLE III

Copper content in Swedish samples of peat soils, ppm dry matter and kg/ha *), in different parts of the country

| Region | Number of samples | Copper ppm | Copper kg/ha |
|---|---|---|---|
| 61°–67° "Norrland" | 60 | 6 (2–30) | 5 (1–52) |
| 59°–61° "Svealand" | 251 | 22 (4–180) | 24 (3–352) |
| 55°–59° "Götaland" | 53 | 16 (4–50) | 20 (2–76) |

*) Kg/ha = kilogrammes per hectare.

A brief comparison of the figures for hay and for soil samples from different parts of the country gives some idea as to a correlation between the copper content in peat soils and in their vegetation. Low copper values in the soil bring about, almost regularly, a depression of the copper content in the vegetation below normal values. In Table IV and in the corresponding Fig. 2 are given combined values for Cu in the soils, calculated as kg/ha, and the values for Cu in the vegetation on the same soils. It is clearly seen that the mean copper content in the vegetation rises very rapidly with increasing values for copper in the soil, until normal values for the vegetation are attained, which occurs when the copper content of the soil corresponds to about 15 kg/ha. When the soil contains only 5–10 kg copper per ha, or less, down to a depth of 20 cm, there is an obvious risk of a vegetation with a deficiency in this metal. On the other hand, an increase of copper in the soil to much higher values than about 20 kg/ha does not bring about a corresponding increase in the copper content of the vegetation. Thus as a rule, peat soils with a

TABLE IV

The relation between copper content in peat soils and in their vegetation. Mean values and variations

| Copper in the soil kg/ha | Number of soils with vegetation | Copper in the vegetation | | |
|---|---|---|---|---|
| | | Cu, mean ppm | Variations | Number of values below 3.5 ppm |
| 1– 2 | 14 | 2.4 | 1.0–3.5 | 12 (86%) |
| 3– 5 | 38 | 2.9 | 1.4–5.3 | 27 (84%) |
| 6– 10 | 4 | 4.8 | 3.3–6.0 | 1 (25%) |
| 11– 20 | 13 | 5.3 | 3.3–7.2 | 1 (8%) |
| 21– 50 | 6 | 5.6 | 3.3–8.3 | 1 |
| 51–100 | 3 | 6.1 | 4.8–7.0 | 0 |
| over 100 | 11 | 6.8 | 5.2–9.2 | 0 |

content of copper corresponding to 50 150 kg/ha, or even more, support a vegetation with quite a normal copper content.

In this respect, however, individual pairs of analyses of soil and vegetation may differ appreciably from each other and from the mean curve as in Fig. 2. This is especially the case with soils deficient in the metal in question, where such anomalies in the copper content of the vegetation are often met with, in analyses made in different years. As a result of different conditions from year to year in a soil poor in copper, the copper content in the vegetation may show variations within very wide limits. In a series of long-term investigations on the vegetation on a soil clearly deficient in copper, many

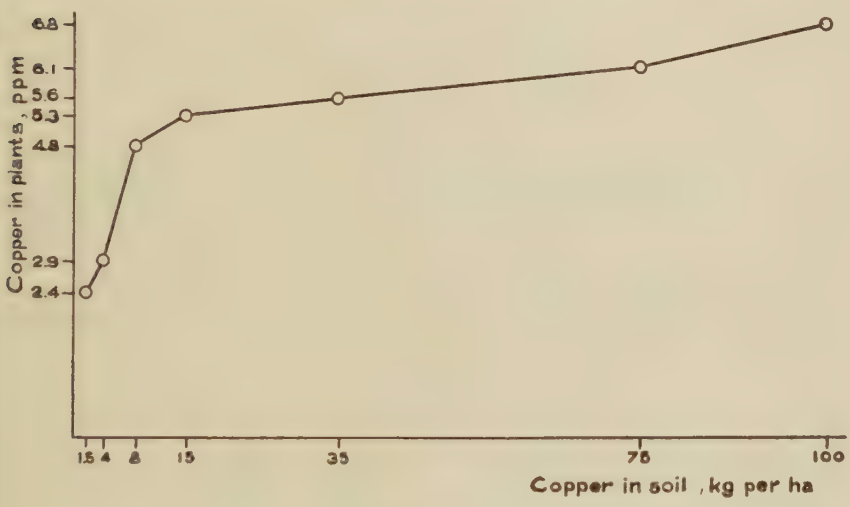


Fig. 2

results of this kind were obtained, a number of which are collected in Table V and Fig. 3.

From the appearance of the curve in Fig. 2 it is quite clear, that even very small differences in the availability of the copper and in its fixation in soils poor in this metal, may bring about much greater differences in the copper content of the plants than may be observed on soils with a higher content of the metal.

The variations from year to year given in Table V and in Fig. 3, demonstrate at the same time that it may be regarded as almost impossible to settle by means of plant analyses only, whether a soil

TABLE V

Yearly variations in the copper content of grass vegetation on peat soils. very poor in copper

| | Year | Cu in plants ppm | | Year | Cu in plants ppm |
|---|---|---|---|---|---|
| Exp. 503 Gisselås | 1941 | 4.0 | Exp 507 Gisselås | 1942 | 2.2 |
| | 1942 | 2.9 | | 1943 | 2.3 |
| | 1943 | 2.5 | | 1944 | 4.2 |
| | 1944 | 1.7 | | 1945 | 3.5 |
| | 1945 | 2.6 | | 1946 | 3.6 |
| | 1946 | 4.4 | | 1947 | 3.4 |
| Exp. 504 Gisselås | 1941 | 3.4 | Exp. 511/I Gisselås | 1942 | 4.5 |
| | 1942 | — | | 1943 | 1.4 |
| | 1943 | 1.8 | | 1944 | 4.5 |
| | 1944 | 1.5 | | 1945 | 3.2 |
| | 1945 | 2.0 | | 1946 | 2.0 |
| | 1946 | 2.8 | | 1947 | 2.1 |

requires fertilizing with copper in order to obtain crops with a normal content of the metal. The results of such analyses may, in one year, show that the supply of copper is nearly normal, whereas in the next year a demand for an addition of copper is clearly demonstrated. In such a case, of course, the risk of crops with an unsatisfied demand for copper always does exist, but it does not neces-

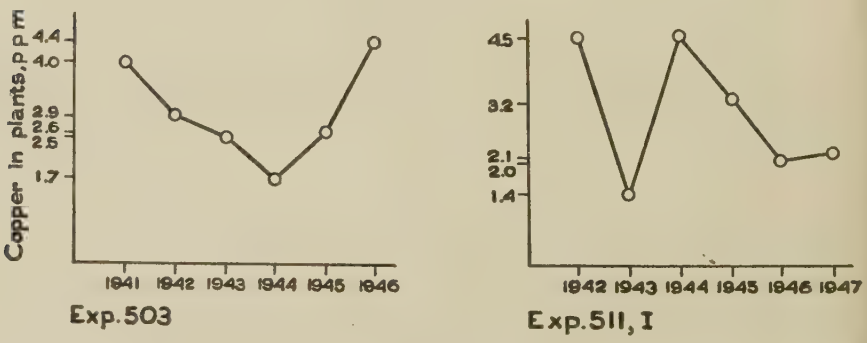


Fig. 3

sarily manifest itself more frequently than perhaps during two years or a single year over a period of about five years. As the leaching of the copper with water is almost nil from soils with satisfactory pH-values, such as will be demonstrated later, soil analysis is a much better indicator of the copper status. It is, of course, impossible to

state from a soil analysis whether the soil will give rise to a copper-deficient vegetation in one of the succeeding years, but it gives some idea of the risk of this situation occurring.

Analytical observations concerning the variability of the copper content of crops from year to year on soils deficient in this metal, correspond clearly to practical experience and to fertilizing experiments on soils where the risk of white tip disease and a reduced grain yield is prevalent. Where losses of this kind are observed, they do not occur regularly every year when grain crops are grown on the soils in question. Frequently a deficiency in copper appears rather surprisingly on soils where losses of this kind were not met with earlier.

On soils of this kind it was found that even fertilization with a rather small quantity of copper sulphate may result in the annual variation in copper content of the crop vanishing (Tab. VI). For this

TABLE VI

Results of a long-term fertilizing experiment on a peat soil, poor in copper. Cu-content in ppm dry weight of hay (grasses only). Fertilized by top-dressing in 1941

| Fertilizing copper sulphate kg per ha | 1942 | 1943 | 1944 | 1945 | 1946 |
|---|---|---|---|---|---|
| Control = 0 | 2.9 | 2.2 | 1.7 | 2.3 | 3.6 |
| 5 | 3.7 | 3.6 | 3.7 | 3.3 | 3.8 |
| 25 | 5.9 | 4.1 | 5.3 | 5.2 | 5.5 |
| 125 | 4.8 | 5.4 | 6.2 | 8.2 | 7.2 |
| 250 | 5.4 | 5.4 | 7.8 | 8.1 | 9.2 |

reason, we may consider only the effect of the smallest supply i.e. 5 kg copper sulphate per hectare in the table, in comparison with the copper content of the crops on the control plots. From the first and the second line in the table it may be concluded that a great annual variation exists in the availability of copper in the control plots, but that this variation is almost completely suppressed by a very small application of copper sulphate. The copper content of the soil in the controls was about 3 kg/ha; thus when supplied with 5 kg copper sulphate 4.25 kg/ha.

In the field experiment, summarized in table VI, the copper salt was applied as a top dressing in the spring of the year 1941. The effect of different additions of Cu to the soil on the copper content in the plants is that of a rather continuous increase. A gradual progressive fixation of the metal in the soil does not take place. About 40–50 kg of copper sulphate per hectare would probably be a suit-

able quantity on this soil to bring about normal copper conditions in the soil and in the vegetation for a number of years to come. This addition, again, is enough to produce a total amount of about 18–20 kg copper per hectare down to a depth of 20 cm in agreement with the conclusions drawn from Table IV and Fig. 2.

For reasons already mentioned, it is not to be expected that one may get a good idea of the copper requirement of a soil, deficient in this element, by means of analyses of the vegetation only. Greater reliability is to be expected using soil analyses for this purpose. In this respect, however, it is a matter of interest whether the best method is to determine, as is the case in this work, the total copper by wet combustion of the soil in the same manner as for the samples of the vegetation, or whether it may be worth while seeking an extraction method, indicating the most soluble ("plant-available") fraction of the copper. Difficulties would arise, however, even if it was really possible to find a method which would reliably determine the plant-available fraction of the copper in the soil. It seems us quite clear that the figures obtained by means of such a method would suffer from a similar drawback to that mentioned above in the case of plant analyses. They would show the same variations as the copper in the plants from one year to another. Such analyses, therefore, would only give an idea of the temporary situation in the soil with regard to plant-available copper, a situation that, by testimony of the vegetation itself, is of little value from a practical point of view. For this reason the authors find that soil analyses made according to the method used in this investigation are more applicable in the case of this minor element. This is supported by the fact that, in practice, a deficiency of copper in the crops in Swedish farming, is observed almost exclusively on soils, where the amount of copper, determined by the method described, is really smaller than that corresponding to about 15–20 kg of the metal per hectare down to a depth of 20 cm. An exception to this rule will be mentioned later on.

A number of field experiments, such as the one recorded in Table VI, were studied six years after top dressing with copper sulphate in relation to the copper content of the soil at different depths down the soil profile. The figures thus obtained, corresponding with the experiment recorded in Table VI, are collected in Table VII.

The analyses show clearly that the migration of the copper down into the soil is extremely slow. In the case of 25 kg copper sulphate

TABLE VII

Copper content, ppm dry matter in a peat soil, at different depths beneath the surface, six years after top-dressing with different amounts of copper.sulphate

| Depths beneath soil surface cm | Control, without addition of Cu | 25 kg copper sulphate/ha | 250 kg copper sulphate/ha |
|---|---|---|---|
| 0– 5 | 7.0 | 93 | 1600 |
| 5–10 | 5.8 | 5.8 | 8.6 |
| 10–16 | 6.1 | 5.6 | 5.5 |
| 16–24 | 5.9 | 4.7 | 4.5 |
| 24–36 | 5.5 | 3.8 | 4.2 |

per hectare none at all, and in the case of 250 kg per hectare only a very small amount, corresponding to about 0.2% of the quantity supplied as a top dressing could be detected at depths lower than 5 cm beneath the soil surface six years after application. The results of a series of similar field experiments give rise to the same conclusions, i.e. the effect of the top-dressed copper on the copper content of the plants during the next 2–3 years may be rather small and the mobility of the copper ions down into the soil is either also very small or impossible to detect. Nevertheless, the fertilization of a Swedish peat soil with copper to a total copper content of 15–20 kg per hectare brings about, after some time, a vegetation which is normal as regards its content of this metal. The fixation of copper in the upper layers of a peat soil is consequently very marked, but it does not appear to prevent the crops from taking up a sufficient amount, provided that the total copper content of the soil in the rhizosphere does not fall below a critical value. In the experiments of the authors this critical value corresponds to 15 20 kg per hectare in the upper 20 cm of the soil.

The slow downward migration of copper ions from the surface of the soil indicates that copper fertilizers should not be used as a top dressing. Copper fertilizers may be ploughed in to ensure a rapid action. The authors have obtained good results in experiments of this kind on copper-deficient soils. Cereals responded immediately to such fertilization by a marked increase in yield and a better quality of the grain. At the same time the copper content of the vegetation became normal.

IV. *Analyses and experiments on mineral soils.* Mineral soils, poor in copper, exist in Sweden to a rather great extent. While peat soils, poor in this metal, are most common in the northern part of the

country, mineral soils of this kind have attracted attention mostly in the southern part of Sweden. Especially between the 56° and the 57° parallels rather large areas of sandy and other light mineral soils produce a vegetation with a reduced content of copper. At the same time these soils contain comparatively small quantities of this element per hectare, determined in the way already described. These observations seem to point out, that in Swedish mineral soils, as well as in peat soils, a real deficiency in the metal is a matter of greater interest than a fixation of the metal by soil constituents.

In other parts of the country, also, there occur mineral soils deficient in copper, but deficiencies are not such a general problem as in the southern parts. Nor are they common when compared with peat soils in the North of Sweden.

Clay soils, as a rule, do not show a markedly reduced copper content in the vegetation or in the soil itself. Large areas of clay soils in middle Sweden have, rather regularly, a copper content, amounting to about 90 kg of the metal per hectare in the uppermost 20 cm. In the South, however, the clay soils are seldom so rich in this metal as indicated by this figure, but at the same time they are affected by a shortage in copper to a much lesser degree than sandy soils, just as in the case of other biogenic elements such as Mg, K and others. Mineral soils in Sweden have, on the average, about the same content of copper as peaty soils, expressed as ppm by weight, but calculated as kg copper per hectare the mineral soils, as a result of their greater specific gravity, are much richer with a mean value of 44 kg per hectare as against 20 kg per hectare for the peat soils.

Copper deficiency occurs in some types of assorted mineral soils (esp. sandy soils) and in moraine soils, the former being at times rather rich in lime and the latter as a rule more acid with a pH between 5 and 5.5 or even lower.

Table VIII gives an abbreviated account of the copper content of Swedish samples of hay from mineral soils in different parts of the country, whilst Table IX gives the copper values for the soils.

Comparisons of the copper content in the mineral soils and in the plants give rise to exactly the same conclusions as those described earlier in the case of peat soils. The lowest copper contents found for peat soils expressed as kg metal per hectare down to a 20 cm depth have no equivalents among the mineral soils with their larger specific gravity. On the other hand, the curve (Tab. X and Fig. 4), illustrat-

TABLE VIII

Copper content in Swedish samples of hay from mineral soils, ppm dry matter, in different parts of the country

| Region | Number of samples | Copper, ppm | |
|---|---|---|---|
| | | Mean | Variations |
| 61°–67° "Norrland" | 68 | 6.2 | 2–13 |
| 59°–61° "Svealand" | 200 | 7.3 | 3–13 |
| 55°–59° "Götaland" | 250 | 5.8 | 2–14 |

TABLE IX

Copper content in Sewdish samples of mineral soils, ppm dry matter and kg/ha, in different parts of the country

| Region | Number of samples | Copper ppm | Copper kg/ha |
|---|---|---|---|
| 61°–67° "Norrland" | 31 | 13 (4–25) | 33 (11–73) |
| 59°–61° "Svealand" | 108 | 34 (4–150) | 93 (11–424) |
| 55°–59° "Götaland" | 240 | 9 (2–90) | 23 (4–252) |

ing the relationship between the copper contents in mineral soils and in their vegetation, is of the same shape as that in the case of peat soils (compare Fig. 2). There seems to be, however, a slight difference between the effectiveness of the copper (determined by the method used by the authors) in the case of peat soils and in the case of mineral soils. The copper in the mineral soils does not appear to be quite so easily available to the plants. Whilst 15 kg Cu per hectare in peat soils appears to be sufficient for a normal composition of the vegetation, the corresponding minimum Cu-content in a mineral soil must be slightly higher, i.e. about 20 kg/ha.

TABLE X

The relation between copper content in mineral soils and in their vegetation. Mean values and variations

| Copper in the soil kg/ha | Number of soils with vegetation | Copper in the vegetation | | |
|---|---|---|---|---|
| | | Cu, mean ppm | Variations | Number of values below 3.5 ppm |
| 6– 10 | 6 | 2.3 | 1.6–3.6 | 5 (83%) |
| 11– 20 | 16 | 3.6 | 1.7–4.5 | 9 (56%) |
| 21– 30 | 7 | 6.6 | 5.0–9.2 | 0 |
| 31– 50 | 12 | 6.5 | 3.5–8.0 | 0 |
| 51–100 | 6 | 7.3 | 4.8–11.5 | 0 |

Fertilizing experiments in the field with copper sulphate, in the same quantities as used on peat soils, also indicate a difference

between mineral and peat soils. If the risk of fixation of this metal be greater in the organic matter than in the outer layers of the mineral

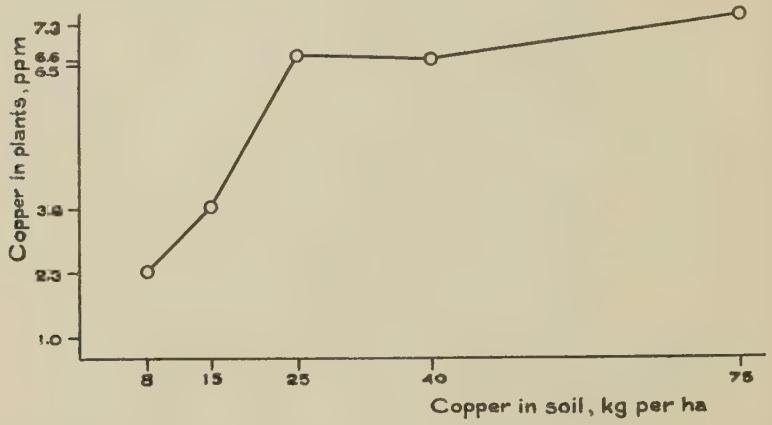


Fig. 4

particles in the soil, it must certainly be expected that long-term fertilizing experiments with copper sulphate as a top dressing should be more effective with regard to the copper content in the vegetation on mineral soils than is the case on peat soils. According to the authors' experiences this however, is not really the case. Table XI

TABLE XI

Results of a long-term fertilizing experiment on a mineral soil, poor in copper. Cu-content, ppm dry weight of hay (grasses only) Fertilized by top-dressing in 1942

| Fertilizing copper sulphate kg per ha | 1943 | 1944 | 1945 | 1946 | 1947 |
|---|---|---|---|---|---|
| Control = 0 | 3.8 | 3.0 | 3.1 | 3.0 | 3.9 |
| 5 | 4.5 | 3.5 | 3.4 | 3.1 | 4.2 |
| 25 | 4.0 | 3.8 | 3.5 | 3.3 | 4.8 |
| 125 | 5.1 | 4.1 | 3.5 | 3.7 | 5.8 |
| 250 | 5.6 | 4.5 | 4.1 | 4.0 | 5.6 |

shows the results of a fertilizing trial with copper sulphate in the field on a sandy soil, on the same farm (Gisselås) as the experiment on peat soil recorded in Table VI.

It is observed from the table, that top dressing with copper sulphate on a mineral soil gave rise to a much slower increase in the copper content of the harvest than was the case in a similar experi-

ment on a peat soil. Compared with the corresponding observations on the peat soil, the leaching of the copper down into the mineral soil, however, shows a slightly faster migration (Table XII).

TABLE XII

Copper content, ppm dry matter in a mineral soil, at different depths beneath the surface, five years after top-dressing with different amounts of copper sulphate

| Depth beneath soil surface cm | Control, without addition in Cu | 25 kg copper sulphate /ha | 250 kg copper sulphate/ha |
|---|---|---|---|
| 0–5 | 5.7 | 13.6 | 180 |
| 5–10 | 4.9 | 4.3 | 6.1 |
| 10–16 | 4.1 | 3.7 | 5.7 |
| 16–24 | 2.0 | 3.1 | 5.7 |

The conclusions drawn from these comparisons of field experiments on a peat soil and on a mineral soil are as follows.

In the peat soil the fixation of copper, as measured by its availability to the plants, was distinctly less pronounced than in the mineral soil. On the peat soil, a top dressing of 25–125 kg of copper sulphate per hectare brought about progressively increasing figures up to normal values, whereas on the mineral soil a much larger quantity (250 kg) was unable to give this effect in 1944–46. Nevertheless, the migration of copper down into the soil, was slightly more rapid in the mineral than in the peat soil. Thus the copper, fixed chemically in the peat, seems to be more available to the plants than copper, fixed in the mineral particles.

It may be noted that the fertilizing experiments, described above, were carried out on soils with sufficient natural lime and pH-values in KCl slightly above 5.

V. *Further comparisons between the effect on the yield, of fertilizing with copper sulphate on peat soils and on mineral soils with different contents of organic matter.* The results obtained in the long-term experiments now described are strongly supported by a number of short-term fertilizing experiments in Sweden with different crops and on different soils in the southern parts of the country. The total number of these experiments, made over a number of years, was 141, and they are accounted for in Table XIII. The results are divided into groups, according to the type of soil and its content of copper, calculated as kg per hectare.

TABLE XIII

Results of fertilizing experiments with copper sulphate on Swedish soils with different content of organic matter and copper

| Loss on ignition | Peat soils 21–90% | | | Mineral soils 6–20% | | | Mineral soils 2–5% | | |
|---|---|---|---|---|---|---|---|---|---|
| Copper kg/ha | <15 | 15–30 | >30 | <15 | 15–30 | >30 | <15 | 15–30 | >30 |
| Number of Experiments | 15 | 11 | 3 | 24 | 26 | 18 | 12 | 20 | 10 |
| Effect, % of yield, mean | + 11.4 | +4.9 | +1.7 | +3.4 | +0.3 | +0.4 | +4.2 | —0.2 | —0.1 |

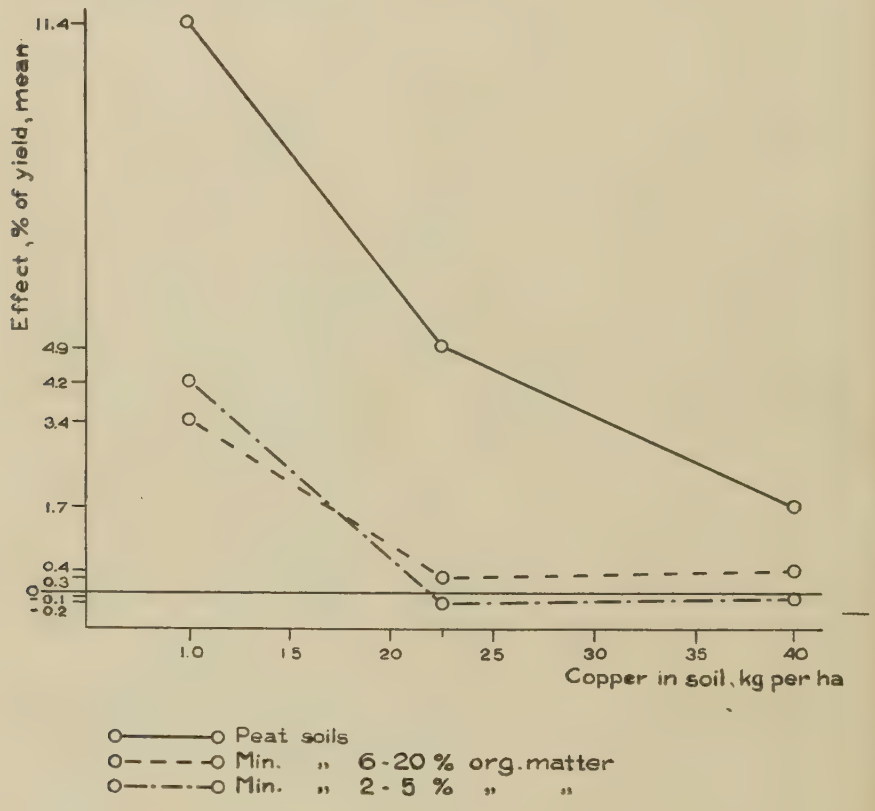


Fig. 5

The results may be summarized as follows.

On peat soils, fertilizing with copper sulphate has on the average a greater effect, than on mineral soils with the same copper content. On mineral soils the action of a copper dressing appears to be

diminished by the mineral material. Another explanation would be that the organic matter of mineral soils is better able to fix the metal than is the organic matter in peat soils. That the organic matter in a mineral soil, however, is of relatively small significance in the availability of copper to the plants, may be concluded from the observation that there is little or no difference between the results on mineral soils with different contents of organic matter.

An exception to the rule, that the copper content of the soil (as determined by the method described in this paper) is able to give some idea as to the requirement for fertilizing with copper, was found in the gyttja soils.

It may be necessary to explain here, the meaning of the word gyttja though it is used internationality in the geological literature. The Swedish word "gyttja" was adopted for scientific purposes by H a m p u s v o n P o s t to indicate more or less organogenic lacustrine and marine sediments formed in waters, rich in plant nutrients. Gyttja consists of detritus from the animals and plants in the water and remains of pollen, spores and plankton organisms, with an admixture of various kinds and amounts of mineral particles. In some types of gyttja there is also much detritus from trees, shrubs and plants that lived on the margins of the water. Different types of gyttja are named after their most conspicuous ingredients: silty gyttja, clayey gyttja, calcareous gyttja, detritus-gyttja, algae-gyttja etc. Sometimes a group, or even a single genus, of algae may be a main constituent: diatom-gyttja, *Vaucheria*-gyttja.

Being mainly an anaerobic process, the formation of gyttja leaves the chlorophyll more or less unchanged. The chlorophyll may be extracted and detected in different ways in the extracts. This is a means of distinguishing between peat and gyttja. A far easier way however, is a direct microscopical survey of a small soil sample treated with a little 10% alkali soln. This method also enables one to estimate the relative amounts by volume of different constituents both in peat and gyttja.

In Sweden, cultivated gyttja soils are found in different parts of the country. They abound in the regions of the large lakes, esp. Mälaren and Hjälmaren, and on the south-eastern coast, by the Baltic. As a rule, gyttja soils form good arable land. In some districts, however, several gyttja soils have a relatively high content of sulfides and therefore a large lime requirement.

Gyttja soils may have a copper content which exceeds 25 kg per hectare, of about 35 -60 kg ha, but nevertheless show significant increases in the yields of grain after fertilizing with copper sulphate. It seems quite clear that in these gyttja soils there is a fixation problem connected with the original copper content of the soils which, how-

ever, does not influence the effectiveness of copper supplied in fertilizers. On other types of Swedish soils, previously examined in this respect, fertilizing effects on the yield are very rarely met with if the soil has a content of 15–20 kg of this metal per hectare down to a depth of 20 cm.

VI. *Lime and pH in their relation to the availability of copper to plants.* In most papers on this subject, it is stated that the availability of copper decreases with increased alkalinity of the soil. Piper (1942) found that an increase in acidity from pH 8.6 to 4.0 increased the availability somewhat when measured by plant analyses, this dependence, however, being much less marked than that of the availability of manganese. Lucas (1948) reports that the binding of copper in the soil is stronger at higher than at lower pH-values in the pH-region between 3.0 and 7.2. Gaarder and Røyset (1947) state that there is minimum copper assimilation by oats on a moor soil in Norway at about pH 5.5. This statement was made as a result of a number of plots with different copper status. The effects of copper dressings, however, was not greatly affected by variations in pH, and it seems that the function of pH was less significant at higher copper contents. Steenbjerg (1940) points out that there is a minimum solubility of copper in soils at about pH 5.5. Burström and Boratynski (1936) state that the assimilation of copper from water solutions is greater at higher pH-values, this being able to counteract the effects of a lower solubility of the Cu in the soils under these conditions.

Even if there is thus a general opinion that different Ca-levels and different pH-values are able to affect the assimilation of copper by plants, there occur differences as to the effectiveness of these factors under different conditions, which is a matter of practical interest in farming. A rather small effect of lime or pH on the uptake of copper may be neglected in practice of course, but a greater effect should necessitate consideration of the lime status and pH-values.

According to the experience of the authors of this paper, the content of calcium carbonate and the pH-values of the soils do not in practice, interfere to a very great extent with the plant-availability of copper. Under Swedish conditions, soils rich in lime are associated to a much higher degree with deficiency symptoms of manganese than of copper. The ratio copper in soil: copper in vegetation does

not seem to be different, to a marked degree, in the case of calcareous and acid soils.

Leaching of copper from the soils, which appears to be practically negligible (see Table VII and Table XII) in soils in a satisfactory condition as to lime and pH, may become a matter of more interest under very acid soil conditions. As a result, a somewhat higher pH-value, obtained by liming the soil, may be able to protect the soil from losses of this kind and may contribute to better conditions with respect to available copper after a number of years. An example of this kind is described in the following.

Tables XIV and XV demonstrate the relation between the copper content in the vegetation and the pH-values of Swedish soils of two different types. The mineral soils in Table XIV are all to be regarded as satisfactory with respect to plant-available copper, with copper contents (measured by the method used by the authors) between about 30 and 50 kg per hectare. The vegetation analyzed was flax straw. The peat soils in Table XV are all very deficient in this metal with only about 2 kg copper per hectare in the upper 20 cm, and the vegetation , in this case grasses for hay, is also poor in copper.

TABLE XIV

The effect of different pH's of soils on the copper content in the vegetation under similar conditions in other respects Mineral soils, satisfactory as to plant-available copper

| Number of cases | Soil pH (in KCl) | Cu in plants, ppm mean | Cu in plants, ppm variations |
|---|---|---|---|
| 3 | 5.8–7.0 | 6.1 | 5.9–6.2 |
| 8 | 4.0–4.5 | 6.4 | 4.7–9.2 |
| 2 | 3.7–3.9 | 7.1 | 7.1–7.3 |

TABLE XV

The effect of different pH's of soils on the copper content in the vegetation under similar conditions in other respects. Peat soils, deficient as to plant-available copper

| Number of cases | Soil pH (in KCl) | Cu in plants, ppm mean | Cu in plants, ppm variations |
|---|---|---|---|
| 4 | 4.6–5.3 | 2.3 | 1.8–2.8 |
| 3 | 3.9–4.4 | 3.5 | 2.8–4.0 |
| 3 | 3.8 | 3.8 | 2.5–4.7 |

It is seen from both the tables, that as a rule low pH-values, are associated with a somewhat higher copper content in the vegetation

if the examination is carried out on soils with similar contents of copper, this trends being most evident on peat soils, poor in this metal. The results given in Table XVI however, suggest a rather different story. In this case, a rather acid mineral soil was limed in 1930 with 6000 kg CaO per hectare, and soil and vegetation were examined as to their copper status in 1948, i.e. 18 years after liming.

TABLE XVI

Copper content of soil (light clay) and vegetation 2 june 1948 in an experiment with lime at Lanna experimental farm in Sweden. Limed plots with 6 000 kg/ha CaO in 1930. Vegetation: barley at an early stage of development showing significant advantage of lime, visible to the eye. By courtesy of Mr. O. Perman, Lanna

| | Controls | Limed plots |
|---|---|---|
| Dry weight of plants, g/m² | 33,3 ± 2,6 | 66,7 ± 3,9 |
| Soil pH (in KCl) | 3.9 | 4.4 |
| Soil pH (in water) | 5.1 | 5.7 |
| Copper in soil, ppm | 5.6 ± 0.3 | 7.6 ± 0.6 |
| Copper in plants, ppm | 4.9 ± 0.2 | 6.2 ± 0.2 |
| " " " , g/ha | 1.63 (100) | 4.14 (254) |
| Manganese in plants, ppm | 188 ± 18 | 95 ± 15 |
| Yield of corn, kg/ha | 1870 ± 171 | 3960 ± 285 |
| " of straw, kg/ha | 2590 | 3570 |

In this experiment, the copper content of the soil, 18 years after liming, was 35% higher on limed than on unlimed plots. This effect cannot be explained by the copper content of the lime used, Swedish limestones according to our experiences having about the same copper content as the soil in question or even less. At the same time, the copper content of the vegetation was 26% higher on limed than on unlimed plots. As a result of the larger yield on limed plots the total copper content in the vegetation, per hectare, was still further increased by liming to about 250% of the value on unlimed soil. The manganese content of the crop however, large from a physiological point of view on unlimed plots, was diminished on the limed parts of the experiment to somewhat more normal values.

From these figures the conclusion may be drawn, that one may get quite contrasting results concerning the effects of lime on plant-available copper in a soil, if this effect is examined in an experiment of only one year's duration, as compared with a long-term one, and that it is impossible to state whether, under all conditions, a larger lime content in a soil will bring about a decrease in the copper content of the vegetation.

## VIII. *Summary and discussion.*

Under conditions of Swedish farming, poorness or even a pronounced deficiency in copper in the vegetation is prevalent in different parts of the country. This is chiefly the case on peat soils in North Sweden and on sandy and other light mineral soils in the southern part of the country.

Long-term fertilizing experiments and analytical surveys of the copper content of soils and crops have given much information as to the availability and fixation of copper in Swedish soils.

The analytical method used gives the total exchangeable and organically combined copper in a soil. A partial extraction method, extracting only the more readily soluble fraction of the copper in the soil, should of course, give a better correspondence between soil and crop values. But the fact must be taken into consideration that soil copper values obtained in this way might show fluctuations similar to annual variations found in crop analyses, making it impossible to obtain reproducible values for the copper status of a soil.

Preliminary results indicated a relationship between the copper contents of the soils and the vegetation which was investigated further. It was found that the mean copper content of the plants increases very rapidly with increasing copper content of the soil up to a soil value of about 15 kg per hectare for peat soils and about 20 kg per hectare for mineral soils. Above these values the mean increases are rather slow. At a soil value of 15–20 kg/ha, the copper content of the vegetation is generally about normal. As a result of this rapid initial increase and of the great variations found from year to year on deficient soils, the condition of the crop with regard to copper on such soils may depend to a large degree on variations in other adventitious factors. It seems quite certain however, that the risk of a low copper content in the vegetation is by far the greatest when the soil is deficient, and that 15–20 kg copper per hectare is sufficient to eliminate the risk on most soils.

Exceptions to the rule, that 20 kg copper per hectare may exclude the need for fertilizing with copper, were found in gyttja soils. In these soils it is quite clear that the original copper is present in some combination, less available to plants, than in other Swedish soils. This kind of fixation however, does not seem to influence the effectiveness of copper supplied in fertilizers.

The results of fertilizing experiments over a period of six years on copper deficient soils show that the copper content of crops on plots receiving no copper can vary greatly from year to year, e.g. from 1.4 to 5 ppm dry matter, in the same kind of vegetation. These variations are sometimes very similar in neighbouring areas but may also differ widely even in places close to one another. It is therefore impossible to make a reliable estimation of the copper status and requirement of a soil from crop analyses of only one year's duration. In addition, the fact that annual variations can differ widely in places close to one another makes it impossible to attribute them solely to meteorological factors. It may be suggested that the soil microflora influences the amount of copper which can be readily assimilated by the

plants. This explanation is quite compatible with the characteristic nature of the occurrence of deficiency symptoms in field crops as observed in practice.

Comparison of soil and crop values for copper shows that the copper in peat soils is slightly more available than is the copper in mineral soils. This is in direct contradiction to the conception in some of the earlier literature on this subject, that fixation of copper by humus in the soil is the main cause of copper deficiency in crops. According to the opinion of the authors, the main cause is a deficiency in the soil itself. In this as in other respects the humus is beneficial and has no disadvantages from the agricultural point of view.

Long-term fertilizing experiments with copper sulphate, in which the fertilizer was applied as a top-dressing in the first year, show a considerable time lag in the effect on the copper content of the crops. At the same time, copper applied as a top-dressing showed a very slow penetration into the soil. For two or three years such copper dressings may have little or no effect on the vegetation. Even six years after top-dressing grassland with copper sulphate at G i s s e l å s which is in a humid region, practically all the copper added was recovered from the top 5 cm. Below this depth there was a very small increase in the copper content of the soil. The immobility of copper during dry weeks in the summer must therefore be very unfavourable for the crops, and the only effective means of obtaining immediate and definite results in a one-year experiment is to plough in the copper fertilizer.

In agreement with the experience that on an average, the copper content of a mineral soil is less effective for the vegetation than the copper in a peat soil, the effectiveness of copper sulphate as a fertilizer on a mineral soil deficient in this metal, was found to be inferior to the effects obtained on peat soils, as measured by the amount taken up by the vegetation. This indicates that, in spite of the fact that chemical analyses show a greater permeability for copper salt in mineral than in peat soils, there may exist some form of fixation of the copper in mineral soils, poor in this element, which makes the copper added less available to the plants.

Further comparisons between the effects on the yield of fertilizing with copper sulphate on peat and on mineral soils with different contents of organic matter give similar results. On different soils with the same content of copper, calculated as kg per hectare, peat soils on the average give better responses to fertilizing with copper sulphate than do mineral soils. No differences in this respect however, could be detected between mineral soils of different humus content.

It is often stated in the literature on deficiency diseases in plants that copper deficiency is caused by the binding of copper in the soil humus. For the reasons mentioned above we find it impossible to explain the occurrence of white tip disease and a low copper content in the vegetation as a result of a high humus content. It is true that these and other disturbances in the normal copper assimilation are rather common on fairly humus-rich soils, for instance on heathy land reclaimed for agricultural purposes (this is the

reason why white tip disease is also called reclamation disease). This fact however, does not necessarily imply an apparent causal relationship between humus content of the soil and copper deficiency of the crops. The results of our experiments indicate that the main and most common cause of copper deficiency in crops is a soil poor in copper, and that the humus content in the soil is not likely to have any unfavourable effect, on the availability of copper. The fact that soils rich in humus are often also poor in copper may be a coincidence; on the other hand there may be a connexion. If so, the reason why some mineral soils maintain a high humus content may be due to a copper deficiency adversely affecting microbial decomposition of organic substances. A high content of humus can hardly be the *reason* for copper deficiency in crops, but it may be a *result* of copper deficiency in the soil. In Sweden, however, we have found no correlation between the Cu content and the humus content of mineral soils. Some copper deficient mineral soils have been cultivated for rather a short time. This also may be a reason why they have maintained a relatively high humus content.

On most soils with similar copper contents, the availability of this metal decreases with increasing alkalinity and higher pH-values of the soils. Effects of this kind however, may be quantitatively different on different kinds of soils and, above all, of little importance on soils with a good supply of plant-available copper. If a soil is highly acid and losses of copper with drainage water for this resaon are obvious, a suitable addition of lime may prevent leaching of copper, conserve the copper content of the soil and, after a number of years, may contribute to a much better copper status.

Received December 15th, 1948.

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Plant and Soil I, no 4 April 1949

# WATER RELATIONS OF OATS WITH SPECIAL ATTENTION TO THE INFLUENCE OF PERIODS OF DROUGHT

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*Introduction.* In the course of an extensive investigation into the relation between the water supply in the soil and the yield of the crop, which has been undertaken by the workers of the Agricultural Experiment Station and Institute for Soil Research T.N.O. at Groningen, the present writer has collected data for the reaction of oats to the supply of water. The investigation has principally concerned the phenomena which occur when the crop is exposed to serious drought and, in the first place, has had the object of studying what alterations take place in outward appearance of the crop, and especially in the production of dry matter and in the growth in length of leaf and stem. The questions put forward were what injurious influence has a temporary drought on the crop and what are the phenomena connected therewith, and to what extent could the crop supply information about the state of the water in the soil.

On working out the results the collected data appeared to yield some insight into the relation between climatic factors, transpiration and development of the crop.

In the important investigations undertaken by von Seelhorst and his pupils [34]) a method was applied which, in important respects, resembles the one followed in the present investigation. The ample supply of water for a crop grown in pots was changed in those experiments at various times into a very limited one, and the reverse occurred with pots which had been first supplied very moderately with water.

In the present investigation the water supply was not changed continuously but, more in accordance with the variable climate of

the Netherlands, the study was directed to temporary periods of drought of various durations, where the water-content was brought to such a low level that the crop suffered serious damage.

In that part of the investigation which had for its object the study of the direct results of variations in water supply on growth and production of dry matter different water-supplies have been compared. Since previous circumstances to which the crops may have been adapted, may influence growth, crops have been investigated which were grown both under ample and limited water supply.

The influence of climatic factors on transpiration has been investigated.

*Plan of the experiments.* Two experiments were made simultaneously, both with "Eagle" oats in Mitscherlich pots. Experiment No. 1 studied the influence of a change in the water supply on the growth of leaf and stem and on the production of dry matter; experiment No. 2 investigated the influence of periods of drought occurring at different stages of the development on the yield of the crop.

TABLE I

| Design of experiment 1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Original treatment | Water supply | Number of pots observed and harvested | | | | | |
| | | Beginning of exp. 5/6 | Duration of treatment (dates) | | | | End of exp. 5/8 |
| | | | 5-12/6 | 12-19/6 | 19-30/6 | 30/6-12/7 | |
| "wet" series | "very wet" | 4 | 2 | 2 | 2 | 2 | 6 |
| | "wet" | | 4 | 4 | 4 | 4 | |
| | "dry" | | 2 | 2 | 2 | 2 | |
| | "very dry" | | 2 | 2 | 2 | 2 | |
| | "extrem. dry" | | | | | 2 | |
| | | | Beginning of exp. 12/6 | | | | |
| "dry" series | "very wet" | | | 2 | 2 | | 5 |
| | "wet" | | | 2 | 2 | | |
| | "dry" | | 4 | 4 | 4 | | |
| | "very dry" | | | 2 | 2 | | |

Experiment 1 (table I) was concerned with a series with comparatively ample water supply, and with a smaller series with a comparatively limited one (difference 400 g water per pot). After the crop had reached a 4–5 leaf stage, the dry weight of the crop from 4 pots of the

"wet" series was determined. Following this the water level in 2 pots was increased by 400 g ("very wet"), 2 others were reduced by 400 g ("dry") and 2 more were reduced by 800 g ("very dry"). After a week, in which the phenomena appearing were observed and measurements of growth taken, the crop from these 6 pots together with 4 pots in which the supply of water had been left unchanged, were harvested. The same treatment was repeated three times; in the last two cases, however, the period was 11 and 13 days, respectively. In the last group, moreover, 2 pots were exposed to a still more intensive drought ("extremely dry"). Here the quantity of water was 950 g lower.

The pots of the "dry" series were dealt with in a similar way (here the "very wet" pots were obtained by adding 800 g water, with, however, this difference, that the experiment only took place in the second and third of the above-mentioned periods. A number of pots with constant limited or ample water supply were retained for the determination of daily transpiration and of final yield.

In experiment 2 a number of pots were separated weekly, and were exposed for some time to intensive drought by the reduction of the water supply by 850 g so that the crop suffered extensively. According to the experiment plan 8 pots were exposed to drought every 14 days, of which two were exposed for 1, 2, 3 and 4 weeks, respectively. Every 7 days 2 pots were exposed for 2 weeks. (Table II).

TABLE II

Design of experiment 2

Dates for the periods of drought to which the crop was exposed

| Duration of drought in weeks | Dates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | first group | second group | third group | fourth group | fifth group | sixth group | seventh group | eighth group |
| 1 | | 5/6– | | 19/6– | | 3/7– | | 17/7– |
| | | 12/6 | | 26/6 | | 10/7 | | 24/7 |
| 2 | 29/5– | 5/6– | 12/6– | 19/6– | 26/6– | 3/7– | 10/7– | 17/7– |
| | 13/6 | 19/6 | 26/6 | 3/7 | 10/7 | 17/7 | 24/7 | 31/7 |
| 3 | | 5/6– | | 19/6– | | 3/7- | | |
| | | 26/6 | | 10/7 | | 24/7 | | |
| 4 | | 5/6– | | 19/6– | | 3/7– | | |
| | | 3/7 | | 17/7 | | 31/7 | | |

At the end of the period of drought the original water supply corresponding to that of the wet series in experiment 1 was restored. The yield in grain and straw and the composition of the crop (num-

ber of panicles, number of grains per panicle and weight of the grains) was determined.

The soil used was a sandy soil of good quality from Marum (prov. Groningen) which contained 7% humus and was of a favourable acidity (pH 5,65); the content of *P* was sufficient and that of *K* ample. In the "wet" and "dry" series the pots were filled with 7 and 6.6 kg of soil, respectively, which contained 1.43 kg of water in the first series and 1.03 kg in the latter one. This agreed with about 55% and 40% of the moisture-capacity determined in the laboratory. According to S h a n t z [35]) no significance may be attributed to these water contents. In fact it is impossible to maintain a constant water content throughout the experiment. On the contrary a more or less thick layer adjusted to field capacity results from adding water to the surface of the soil.

0.6 g N per pot in the form of ammonium nitrate, 0.6 g $P_2O_5$ as monocalciumphosphate and 0.9 g $K_2O$ as potassium sulphate were mixed in the soil in solution.

In the morning hours the pots were brought up to the fixed weight, the water being spread over the surface, which was covered with a layer of pebbles to reduce evaporation. In periods of high transpiration water was also added in the afternoon. The transpiration of each pot was determined by weighing. When adjusting the water-content account was taken as far as possible of the fresh weight of the crop. As far as the water-content is concerned, the successive periods of drought can be fairly well compared; the climatic conditions, especially as regards the temperature in which the drought prevailed, were, however, different.

The drought periods in experiment No. 2 have mainly been given in whole weeks. But because of uneven transpiration in the various periods this has not always been possible. The deviations, however, may well be neglected.

The pots of experiment No. 1 were put on to wagons, which, when it rained and frequently at night, were run into a greenhouse. The pots of experiment No. 2 were always kept outside. As the summer of 1947 was in the main dry, this has presented few difficulties in the regulation of the water supply. The quantity of water added exceeded by far the quantity of rainwater.

For the investigation of the influence of environmental factors on the daily rate of transpiration, use has been made of the observa-

tions of the meteorological station at Groningen. As the pots were weighed daily in the morning the observations of temperature, relative humidity and wind velocity (in degrees Beaumée) made at 2 and 7 p m and at 8 a m the following day were averaged. Moreover at the Agricultural Experiment Station at Groningen an estimate is made daily of the average degree of cloudiness, which is expressed on a 10-division scale. On this scale the figure 0 means a cloudless sky and the figure 10 a completely covered sky.

*Some notes on the development of the crop.* The oats sown as late as 3 May emerged on 9 May. Shortly after this date the number of plants was reduced to 20. At the first observation (26–27 May) the fourth leaf was in full growth. The growth of the last (8th) leaf ended 20–23 June. The panicles appeared from 27–30 June. On 17 July the growth of the stems was completed, while the crop was fully ripe on the 5 August.

The different treatment of the experiments No. 1 and No. 2 led only to slight differences in development. The plants on the pots which were always kept outside were at the beginning rather fuller, while the quantity of dry matter (especially straw) was also a little larger (86.4 against 85.1 g and the yield of straw 48.5 and 45.3 g respectively). Nevertheless both experiments are quite comparable.

The pots of the "dry" series of experiment No. 1 gave a somewhat irregular growth. A certain retardation of growth was soon observed in comparison with the "wet" pots. The crop developed somewhat thinner and the plants were to a greater extent covered with a waxy layer, so that the colour was of a duller green than that of the luxurious crop grown in the "wet" pots.

The crop of the "dry" pots (exp. 1) headed earlier, but ripened in the normal way. The "dry" series yielded only 67.8 g of dry matter, with however a much more favourable ratio of grain to straw (compare p. 33).

After exposure to intensive drought the crop always showed strong symptoms of wilting, followed by the dying of the greater part of the leaves. The youngest or the two youngest leaves were, however, little or not at all affected. The impression was gained that the damage suffered from drought by a young crop was greater than the damage in a later stage; the older crop being in this respect rather more resistent. Further, it seemed that the

appearance of the crop suffering from drought showed a certain recovery after a few days. The appearance of a crop which had been standing for some weeks in a "very dry" soil seemed comparatively fresh. This may be a result of a more xeromorphic structure of the leaves formed in a later stage (S t å l f e l t [38]). It is possible that here a certain adaptation to the scanty supply of water has occurred. It must also be borne in mind that to these "very dry" pots a small quantity of water has been added daily in order to keep the weight constant. It seems not impossible that the crop in the long run has adapted itself to this small supply of water by forming new roots.

A drought which set in early, followed by a normal water supply led, in contrast with a continuous and comparatively low water supply, to a delayed development. After the restoration of the ample water supply there arose a luxurious growth and, as a result, a delayed emergence of the panicles. Where the damage by drought came a short time later this led, however, to an earlier emergence of the panicles and in these circumstances premature ripening occurred.

Drought during the time when the panicles emerged hindered the growth of the stem. Such a crop remained short. This phenomenon, together with the wilting and premature drying of leaf-tops and whole leaves and the thick waxy layer, form the most striking characteristics of a crop suffering from water deficiency.

*The transpiration in the whole period of growth.* The course of the transpiration of plants with ample and limited water supply, from the commencement of the observations to the end, is plotted against time in Fig. 1. The rate of transpiration appeared to be subject to large fluctuations according to weather conditions. The rate appears to be correlated with the temperature. Apart from this variation, the transpiration rate appeared to increase gradually reaching a maximum about the middle of the development. The magnitude of the transpiration is evidently also determined by the transpiring surface of the crop, which first increases and then diminishes.

In order to get a clear idea of these two factors a further analysis has been made. The influence of the temperature during short periods of the development, in which variations of the transpiring surface are of less importance, has been determined graphically. Use has been made of this provisionally ascertained relation between transpiration and temperature to reduce the rate of the transpira-

tion to a constant temperature. This more accurately ascertained course of transpiration has been used as a correction factor to determine more closely the influence of the temperature. Finally, the now well-established relation between temperature and transpiration was used for a determination of the course which the transpiration would have had under constant temperature. This gives an idea of the transpiration rate of the plants in different periods of development.

*Course of the development of the rate of transpiration.* In Fig. 2 the transpiration is corrected to a constant average daily temperature of 15°. The dots and the line drawn give the transpiration of the pots with ample water supply. In a much clearer way than in Fig. 1 the transpiration appears to increase sharply until, about the middle of June, a pronounced peak is reached. After this follows a rather sharp reduction which later on becomes more gradual. At the end of the development this is again followed by a more rapid decline of the transpiration.

The original increase must be attributed to the development of the leaves. The achievement of a maximum before the growth of the leaf has ended (about 20–23 June) will result from the death of the older leaves and especially of those secondary shoots which had not come to further development. The gradual death of the younger leaves has been responsible for the continual and slow decrease of the transpiration. After the growth of the stems had been completed on 17 July, ripening led to a rapid reduction of the transpiration.

In Fig. 2 a thinner line shows the rate of transpiration of the crop which was grown with a limited water supply. The course of this curve differs from the previous one by the less pronounced peak, which is the result of the small importance of the death of secondary shoots in this case, which had formed in a smaller number, and after this, by a somewhat slower decrease of transpiration. The difference between the two series was temporarily relatively small. This second crop ripened a little sooner, following the earlier reduction in the transpiration rate.

For comparison with the normal, the figure gives also the course in two series, where the normal supply of water is interrupted by a drought maintained for two weeks. In both cases the transpiration was much reduced. After the normal water supply had been restored,

however, a noticeably quick recovery set in, so that two weeks later the transpiration was practically normal.

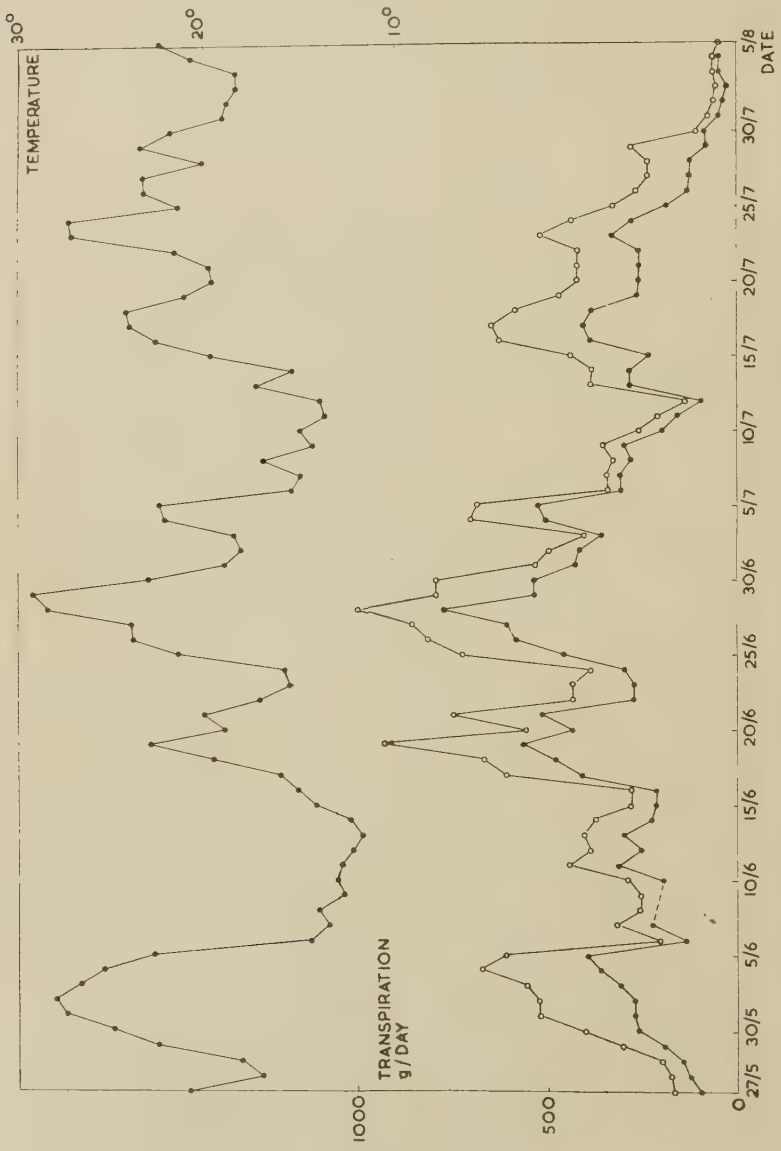


Fig. 1. Below: Course of the transpiration with constant ample (circles) and with constant limited water supply (dots). Above: Course of the average daily temperature.

In the present experiments it is especially notable that the transpiration has already reached a maximum at a comparatively young

stage. In this stage the crop will thus use a great amount of water, especially at a high temperature (a transpiration of $1\frac{1}{2}$ l/day per pot with 20 plants is among the possibilities). A maximum transpiration has been found by Briggs and Shantz [7]) during the emergence of the panicles; thus about two weeks later than in the present experiments. Vidme [44]) and Schmid [31]) have also found that the transpiration is highest in the period shortly before and during the emergence of the panicles. These differences are not, however, of the first importance. The accidental conditions of the experiment will determine when an important reduction of the transpiring surface of the leaves will take place. In the present experiments this occurred more than a week before leaf formation was entirely finished.

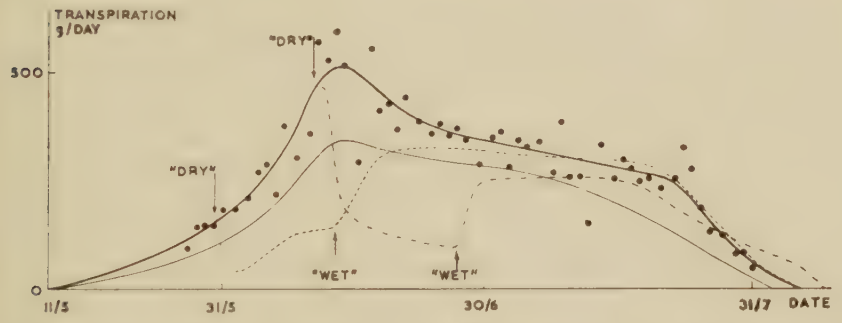


Fig. 2. Course of the transpiration when this is reduced to a temperature of 15°(Thick full line and dots constant ample water supply, thin full line limited water supply. The broken lines give the course with crops which have been exposed to a period of drought of 2 weeks).

*The influence of temperature and air humidity on transpiration.* If the influence of the varying transpiration rate of the plant in the course of the development is eliminated (the transpiration rate at June 8th being taken as normal), the relation between transpiration and climatic factors can be established.

The values obtained with different air humidities have been separated into groups of relative humidities of 46–53, 56–65, 67–75 and 79–80 per cent, respectively and have been plotted separately against the temperature. From the figures thus obtained the influence of relative humidity has been approximately determined. With the aid of this the values in the groups mentioned have been reduced

to relative humidity levels of 50, 60, 70 and 80 per cent, respectively. In Fig. 3 these reduced values have been plotted against the temperature. It appears that the effect of temperature is considerable over the whole range. The influence of air humidity, on the contrary, is only of importance at low temperature. At high temperature the influence is almost negligible.

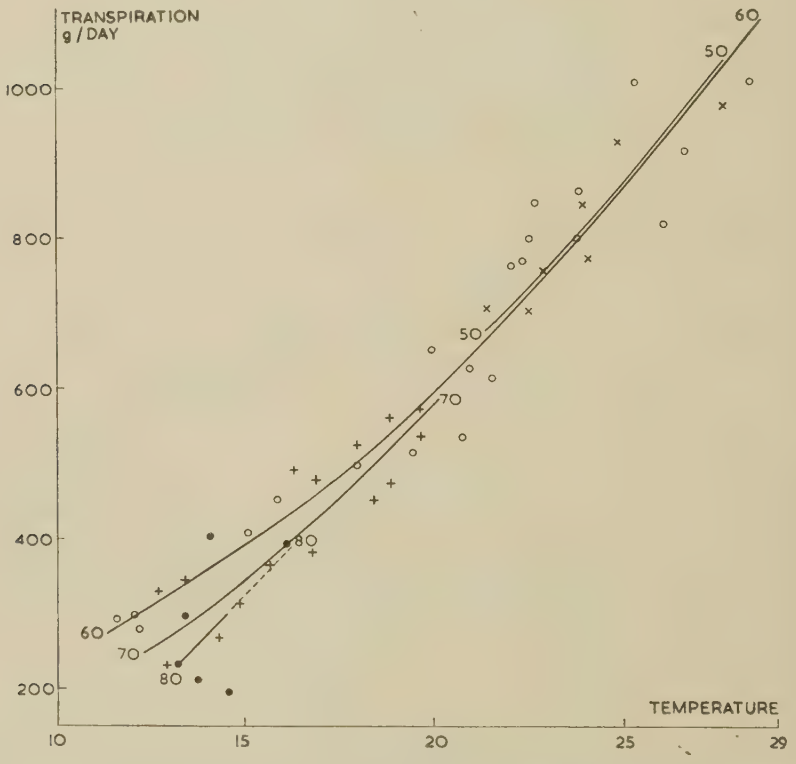


Fig. 3. Relation between transpiration and average daily temperature at different relative humidities. Observations reduced to relative humidities of 50, 60, 70 and 80%, respectively.
× : 50%, ○ : 60%, + : 70% and • : 80% relative humidity.

This result is remarkable. It proves that in the present experiment the transpiration at high temperature has been independent of physical evaporation. The strong influence of temperature indicates that a temperature limited factor in the plant determined the rate of

transpiration. It is, thefore, probable that the transpiration in this case is limited by the absorption of water by the roots which should be dependent on the temperature of the soil. Though the temperature of the soil has not been measured it is likely that a high correlation existed between the average daily temperature of the air and that of the soil in the pots. As the real temperature of the soil is unknown the temperature coefficient has only an approximative value. If allowance is made for the small limiting influence of relative humidity at 15°, in the range of 15–25° $Q_{10}$ appears to be of the order of 2–2¼.

In the recent literature various results are given on the influence of root temperature on transpiration (Döring[12]), Rouschal[29]), Boonstra[4]), Arndt[1]), Kramer[19]) [21]), Clements and Martin[9])). The results, however, point especially to a considerable influence of low temperatures. It may, however, be remarked that the reaction of various plant species is rather different (Döring[12]), Rouschal[29]), Kramer[21])). Besides certainty is lacking whether in these experiments the transpiratory conditions were really optimal in all cases and did not limit the transpiration to some extent. Döring[12]) has mentioned this possibility for explaining the fact that no influence was found above a temperature of 20°. However, clear influences of higher root temperatures on transpiration have been established in some cases. Rouschal[29]) found with *Phaseolus vulgaris* at 20° an almost doubled transpiration rate compared with that at 10°. By extrapolation of the curve it might be derived that higher temperatures would have yielded still higher transpiration rates. Boonstra[4]) established with the same plant for the range of 15–25° values of $Q_{10}$ from 1.3–1.9.

Important indications on the significance of the temperature on the absorption by the roots have been found with experiments on the influence of pump suction on the absorption of decapitated plants. Bode[3]) with sunflowers stated from 10–20° a $Q_{10}$ value of more than 3 and in the range 20–30° a $Q_{10}$ = 2. In agreement with this Kramer[20]) has found in the range 15–25° a $Q_{10}$ = 2.5 with tomatoes and a $Q_{10}$ something less than 2 with sunflowers. According to Köhnlein[18]) the resistance of the roots increases sharply with a lowering of temperature which is of special importance in the case of high transpiration.

Similar influences of the temperature on the water permeability of

the protoplasm have been stated. Krabbe[21]), van Rijsselberghe[30]), Delf[11]), Stiles and Jörgensen[39]), de Haan[14]), found in the range of 10–30° values of $Q_{10}$ between 2 and 3. It is, therefore, probable that the water permeability of the protoplasm of the root cells determines the resistance of the roots. The results of the present investigation indicate that the transpiration at high temperatures is probably almost completely limited by the resistance of the roots. Also at low temperatures the influence is marked. In the latter case the influence of atmospheric condition is also noticeable. The influence of air humidity, however, is much less than has generally been accepted. In this connection it may be mentioned that Tagawa[40]) found with *Phaseolus* that the suction force of the leaf rises to a constant maximum value if the relative humidity of the air at 28° falls below 70 per cent. It is acceptable that in this case the transpiration becomes independent of the air humidity and is limited by the resistance of the roots.

Various authors mention a simple relationship between transpiration and air humidity (Darwin[10]), Henderson[17]), Thut[41]), Martin[24])). Martin[26]) established a different influence of relative humidity at various temperatures. It is, however, plausible that this different effect of temperature may be largely explained by the occurring differences of saturation deficit. Presumably the experiments mentioned have been performed under conditions as in the present investigation only occurred at the lowest temperatures. Moreover, they have partly been carried out with excised leaves.

*The influence of cloudiness and wind on transpiration.* If the conclusion drawn above that the transpiration is limited by the resistance of the roots is correct, an influence of light intensity can only be expected under low temperature conditions. The effect of this factor has therefore been investigated separately within the temperature ranges of 11.6–16.9°, 18.0–21.6° and 22.0–28.4° respectively. This procedure is also necessary because a correlation between light intensity and temperature is apparent (see Fig. 4).

In Fig. 4 the vertical deviations of the dots from the average lines drawn in Fig. 3 are plotted against the degree of cloudiness. In agreement with the expectation a rather clear influence of this factor has only been found at low temperatures. A reduced cloudiness appears to result in an increase of transpiration. Whether this relation is due

to a wider opening of the stomata, to a light effect on the permeability of the protoplasm (as has been suggested in literature), or to a

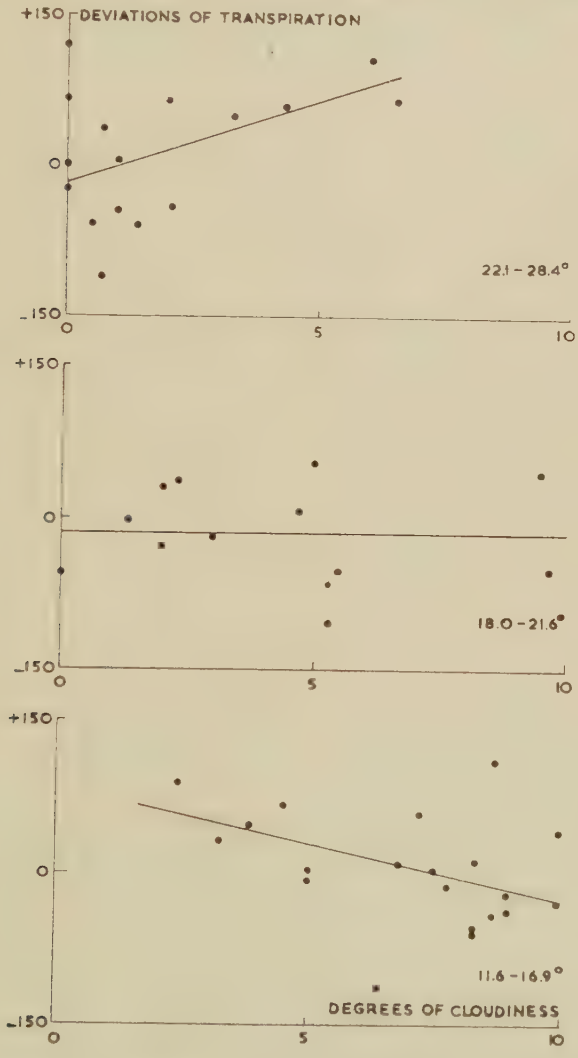


Fig. 4. Influence of cloudiness on transpiration. The vertical deviations of the dots in fig. 3 of the average lines are plotted against the degree of cloudiness at three different average daily temperature ranges.

heating effect on the shoots or on the soil in the pots has, of course, to remain undecided.

No influence of the light intensity has been found at moderate temperature. However, an indication of some limiting effect of full sun shine at high temperature might be seen in the results on very hot days. A partial shutting of the stomata might account for this effect.

Though the measure used for the intensity of the light is not very exact (see p. 307), it is clear that no important part is played by the light factor in the present experiments with oats. Of course this conclusion may not be generalized and does not bear on those experiments, mentioned in literature, where the transpiration is limited by atmospheric factors to a larger extent.

The influence of the wind on transpiration has been investigated in the same way. As a noticeable influence usually only exists when the transpiration is limited by atmospheric conditions, and as Firbas [13]) has stated that the effect of wind at a high transpiration rate differs from that at a low rate, the influence has been studied at various rates of transpiration separately. In no respect, however, has even a slight indication been obtained that the velocity of the wind (which in the present experiments varied between $\frac{1}{3}$ and $4\frac{1}{3}$° Beaufort averages per day), has had any influence.This result generally agrees with those of physiological investigations (e.g. Stålfelt [37]), Firbas [13]), Martin and Clements [27])). The lack of any clear effect in the present exeperiments might, however, be attributed to the dominating influence of the roots, even under conditions which are rather unfavourable for transpiration.

*Ratio between the transpiration with limited and with ample water supply.* In Fig. 5 the ratio between the transpiration with limited and with ample water supply is plotted against time. The average course is represented by a thick full line. The course of this line indicates that the transpiration of the crop with limited water supply increases relatively, but decreases sooner than the crop with ample water supply. The latter is a result of an earlier ripening.

The gradual increase of the ratio in the beginning must be attributed to a relatively stronger production of dry matter per amount of dry matter already formed by the plants grown with limited water supply (see Fig. 5). As a consequence of this the ratio of dry matter is highest at the harvest; being higher for the yield of grain than for the earlier formed straw.

It appears that the ratio of transpiration and that of dry matter is approximately the same (Fig. 5). In other words the transpiration per unit of dry matter is the same in both series, no matter whether limited or ample water is available to the plants. As the amount of dry matter (or fresh matter *)) of a comparatively young crop may be assumed to be a measure of the extent of the transpiring

Fig. 5. Course of the ratio of the transpiration of a crop grown with constantly limited water supply and of a crop grown with constantly ample water supply (dots and full lines). The figure shows further the ratios between the dry matter in both series (+), the ratios between the yields of grain and between the yields of straw in both cases (×), and the course of the average daily temperature (upper line).

surface, one would be inclined to believe that the magnitude of the transpiration is determined by the extent of the transpiring surface. However, it has been claimed before that it is only at low temperature that the transpiration is limited to a relatively small extent by the evaporation from the leaves; at moderate

*) It makes little difference whether in this case the ratio of dry matter or that of fresh matter is taken. The ratio of the latter is somewhat smaller; it amounts to 62, 74 and 70 per cent at 12, 19 and 30 June respectively against 67, 74 and 77 per cent for dry matter (Fig. 5).

or high temperature the ability of the root system to absorb water seems to be the limiting factor. The good correlation does not prove the first mentioned relation to be a direct causal one. It only shows that the amounts of water absorbed by the roots of crops grown for considerable periods of time with different water supplies approximately agree with the amounts of dry matter in the shoots. It will be shown below that a change in the water supply has a considerable influence on the transpiration. It follows from this that a crop grown with limited water supply is more able to absorb water than a crop grown with ample water. That this is really the case follows also from a closer consideration of Fig. 5. A thin full line connects the seperate dots. The course of this line is clearly opposite to that of temperature. This means that the transpiration per unit of dry matter of the crop grown with limited water supply is higher than the average at low temperature and lower than the average at high temperature. The ratio of the transpiration appears to amount in the period of 8–15 June, when the temperature was low, to 70 per cent. The ratio of dry matter was in the same period 67 per cent and that of fresh matter only 62 per cent. Though external factors (air humidity, light intensity) were partly limiting under these circumstances, the transpiration per unit of matter of the crop grown in the "dry"pots appears to be larger.This is only possible if the root system is able to furnish a greater amount of water. That this is no longer the case at a higher temperature, where the resistance of the roots is the only controlling factor, must probably be attributed to the insufficient supply of water in the "dry" series. At high temperature and high transpiration rate presumably the supply of water begins to act as a limiting factor.

Whether the greater ability of the crop grown with limited water supply to absorb water is a consequence of a stronger development of the root system (Tumanow [42]), Schmid [31])), of a smaller resistance of the protoplasm in the roots, or of a higher suction force of the shoots has not been further investigated.

The results obtained agree with those of Veihmeyer and Hendrickson [43]) and of Becket, Blaney and Taylor [2]), who observed that the transpiration of fruit trees over a wide range is independent of the water content of the soil. This has recently been confirmed by Martin [25]) with sunflowers and by Haynes [15]) with corn. These authors concluded that the transpi-

ration should principally be determined by the extent of the transpiring surface and not by the quantity of water available. The divergent results obtained by Lewis, Work and Aldrich[23]) with pear trees on a heavy soil are attributed by the authors to the slow delivery of water by this soil, which is of special importance with a poorly developed root system.

It is, however, probable that in the present investigation the transpiration has not been determined by the extent of the transpiring surface (except for a small extent at low temperature and high humidity), but by the ability of the roots to absorb water. In experiments of long duration there apparently occurs an adaptation to the prevailing conditions. It might be possible that this has also played some part in the experiments of the authors mentioned above.

That a slow delivery of water by the soil as mentioned by Lewis Work and Aldrich[23]) is possibly not unimportant in the present investigation appears from the influence of the temperature on the above-mentioned ratio. The fact that this decreased with a rising temperature, in other words that the transpiration on the dry soil was relatively smaller the higher the temperature, may have been a result of a more difficult delivery of water. The important influence which the temperature, especially at the beginning of the growth has may, perhaps, be due to a less strong root development in this early stage.

*Variation of the water supply and its effect on the rate of transpiration.* When the supply of water of a crop previously grown with a comparatively constant water supply was altered, important changes in the transpiration occurred. All the cases investigated agreed in principle so that the mention of one case will suffice. Fig. 6 gives the transpiration in the period from 19 30 June. The differences with the various treatments were very great and, in particular, the transpiration of the crop on the "very dry" soil was much reduced.

It appears, however, that the difference between the "dry" and "very dry" pots on the one hand and the "wet" and "very wet" on the other hand in the series with previously "wet" soil is *relatively* greater at the beginning of the experiment than later. The difference between "wet" and "very wet" appears, however, to become greater as the experiment proceeds.

In the series with previously "dry" soil the relative difference

between "very dry" and "dry" appears also to decrease. The relative difference between the transpiration of the "dry" on the one hand and the "wet" and "very wet" on the other hand, appears, however, to become greater.

It follows from this that the transpiration with a reduction of the water supply at first decreases and thereafter recovers, but that the transpiration with a raising of the water content increases relatively little in the first few days but later to a larger extent.

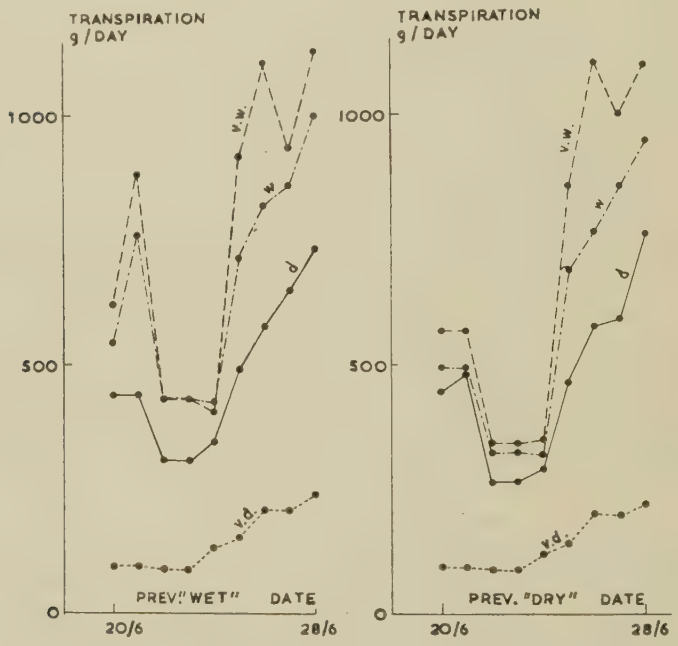


Fig. 6. Transpiration after change of the water supply in "very wet" (v.w.), "wet" (w), "dry" (d) and "very dry" (v.d.), starting from a crop previously grown with ample (left) and with limited water supply respectively (right).

In the first case it may be expected on the ground of the results stated above (Fig. 5), that the transpiration on the drier soil will, in the long run, again approximate to the transpiration on the wetter soil following the adaptation of the roots. In this experiment this has, however, not been further investigated. The rise in the transpiration rate, which appears with an improved water supply, will probably also be of a temporary nature. It might be supposed that this increase is due to a gradual decrease of root resistance. It is, however,

plain that with variations of the water content of the soil of a comparatively short duration, such as often occur with the climate of Holland, and as far as no adaptation to the altered conditions has appeared, *the supply of soil water very largely determines the rate of the transpiration.*

It is interesting to make a comparison between the transpiration of the pots with a similar water supply but with different pre-treatments. Comparing, for example, the transpiration of the crop in the "wet" pots which have previously had the same treatment, with the transpiration of the crop which has previously grown under drier conditions in the experiment of 12–19 June (Table III), then in the first two days on the originally "wet" pots an average transpiration of 372 g was found, and on the originally "dry" pots 272 g. The weight of dry matter, however, in the two series was 17.4 g and 11.7 g respectively, so that the transpiration per gram of dry matter amounted to 21.4 g and 23.2 g respectively. Already at the beginning of the experiment the transpiration in the latter case was 8% higher. Five days later(average of the transpiration the 6th and 7th day) this amount had risen to 21%. The same was observed in the experiment of 19–30 June. In the first two days the transpiration in the previously dry series was 1% higher and in the last two days 16% higher.

TABLE III

Influence of pre-treatment on transpiration

| pre-treatment | treatment | transp. g/day | | air-dried matter | | transp./dry matter | | difference % | |
|---|---|---|---|---|---|---|---|---|---|
| | | 13–14/6 | 18–19/6 | 12/6 | 19/6 | 13–14/6 | 18–19/6 | 13–14/6 | 18–19/6 |
| "wet" | "wet" | 372 | 762 | 17.4 | 29.2 | 21.4 | 26.1 | | |
| "dry" | "wet" | 272 | 730 | 11.7 | 23.0 | 23.2 | 31.7 | 8 | 21 |
| | | 20–21/6 | 27–28/6 | 19/6 | 30/6 | 20–21/6 | 27–28/6 | 20–21/6 | 27–28/6 |
| "wet" | "wet" | 654 | 928 | 29.2 | 52.3 | 22.4 | 17.7 | | |
| "dry" | "wet" | 493 | 899 | 21.7 | 43.6 | 22.7 | 20.6 | 1 | 16 |

The rate of transpiration of the crop which had been previously adapted to a better water supply appeared to be considerably less than the crop grown originally in "dry" soil. In the first two days of the experiment of 12–19 June the transpiration rate calculated on dry matter was 40% and, in the last two days, it was 13% lower. In the experiment of 19–30 June 29% and 12% reductions respectively,

were measured. The originally "dry" and "wet" treated crops differed markedly in appearance. While the leaf of the former was always turgescent, the leaf of the latter was often somewhat wilted. After a few days, however, the crop recovered.

Also with the "very dry" pots the damage was greater when the crop had grown previously on "wet" soil instead of on already rather "dry" soils.

From the comparisons made is appears that the ability to absorb water is markedly greater with a crop grown with a relatively low water supply. Similar results have been obtained by Tumanow[42]). According to this investigator sunflower and buckwheat grown on dry soil or exposed successively at various times to wilting transpire significantly more with ample water supply than do the plants grown constantly with ample water. It is true that Martin[24]) was not able to confirm these results. The first mentioned author established moreover that such plants show a more vigorous resistance to wilting and that the consequences of this become less harmful for the development of the plants.

Finally it should be noticed that it is not unlikely that the observed recovery on dry soil may have to be attributed to the special conditions of a pot experiment. It must be borne in mind that comparatively small quantities of water have been added daily. The surface of the soil remained always wet (Shantz[35])), which may have stimulated the forming of roots and the absorption of water.

*Consumption of water in relation to the production of dry matter.* The quantity of air-dried matter of the shoots amounted to 85.1 g in the "wet" series and to 67.8 g in the "dry". The amount of water transpired per pot in the period of observation amounted to 29.4 kg in the "wet" series and to 19.9 kg in the "dry". If we allow for a small amount (calculated by extrapolation) for the earliest period, then this will be about 30.2 kg and 20.4 kg respectively. In this the evaporation from the soil, which was reduced by a layer of pebbles, is included. It may be assumed that the last named loss with the pots which were kept "wet" will also have been somewhat greater than with the "dry", so that the ratio of the transpiration in both cases probably will have been but little influenced by this. For the forming of 1 kg of air-dried matter 352 kg and 299 kg respectively of water in both series would have been necessary. For absolutely dry matter these

quantities may be estimated at 18% higher, and thus amount to 415 kg and 353 kg respectively. These figures do not differ much from

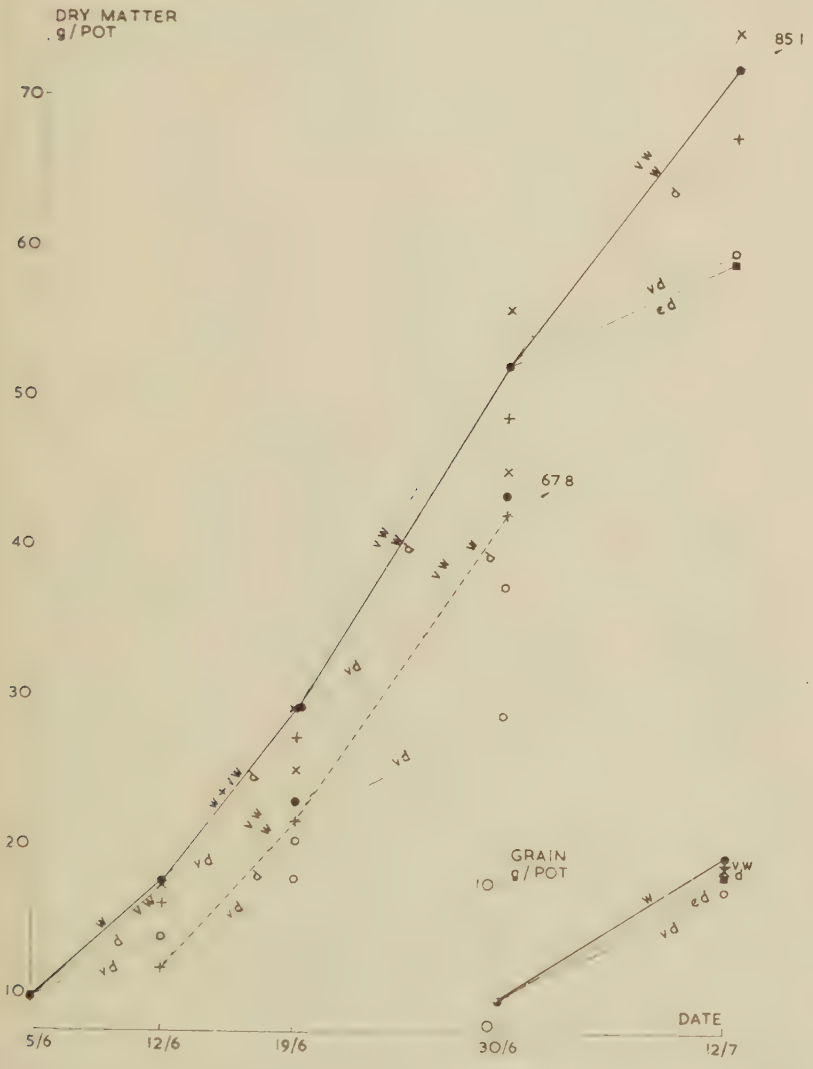


Fig. 7. Course of the production of dry matter after a change of a previously constant water supply, starting from a crop grown with ample water supply (full lines) and of a crop grown with limited water supply (broken lines). Below (right) the formation of grain with different water supply.

each other, but point to a more economical consumption of water on the "dry" soil. The quantities are lower than those given by Briggs and Shantz [7]) for oats namely 597 kg as an average for three years, but do not deviate in an appreciable measure from the results of older investigators, gathered by these authors [6]) in their survey of the old literature (namely 401–665 kg), and from results obtained by Tulaikov (see Maximow [28])) during subsequent years (280–618 kg). The relatively low values obtained in the present investigation may be connected with the hot and dry weather conditions in the year 1947 (Maximow [28])). The quantity of air-dried matter formed per kg of transpired water amounts, in the present investigation, to 2.8 g and 3.3 g, respectively.

The course of the production of dry matter with the "wet" and the "dry" series and the influence of variations of the water supply are shown in Fig. 7. The yields of the "wet" series determined over a longer period show a normal, weak S-shaped growth curve. The growth curve of the "dry" series lying on a lower level runs almost parallel with this. Absolutely, the arrears in the later stages become but little greater, and relatively they become smaller.

The difference between the "wet" and the "dry" series is thus already determined at an early stage and, as can be derived from the ratio of the transpiration (Fig. 5), it has probably already been present at the time of germination.

A more ample water supply in the "wet" series has shown no difference in the first two periods of observation. In the last two periods this has had a very slight favourable influence on the production of dry matter. A smaller water supply ("dry") has, in all periods, resulted in a smaller production of dry matter; in absolute measure the originally "wet" pots have produced not more and in relative measure less than the originally "dry" pots (see Fig. 8). A considerable fall in production is found with a serious drought ("very dry") although the quantity of dry matter produced, taking into consideration the damage suffered, is still measurable. Also with an extreme drought dry matter is still formed in the last observation period. It is noticeable that the dry matter formed under these conditions has been used entirely for the formation of grains. With a severe drought at this stage this is only slightly smaller than with a more ample supply of water (Fig. 7 below-right). Here there appears a premature ripening.

With the "dry" series the considerable production of dry matter, which with a more ample water supply (especially "very wet") has been obtained, is most marked. Under these conditions the production of dry matter, even in an absolute measure is considerably greater than with a crop which has grown originally with a more

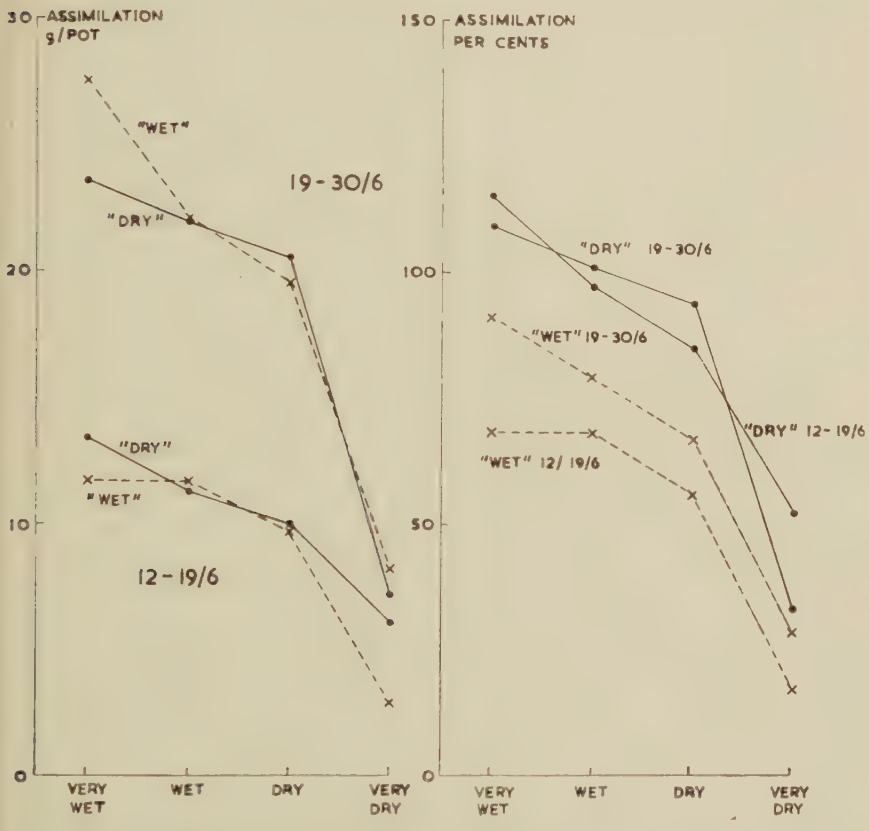


Fig. 8. Production of dry matter in two periods after a change of the water supply (previously "wet" and previously "dry"); left in grams, right in percentages of the amounts present at the beginning of the periods.

ample water supply. A relatively higher production has also been observed in the second observation period, although to a less degree. Similar results with buckwheat were obtained by T u m a n o w [42]), who even found a doubling of the assimilatory intensity under the same conditions. From this it appears that arrears which have arisen on a comparatively "dry" soil can, to a great extent, be made up if

there is plenty of water available. This phenomenon occurs especially in a young crop; it can be observed fairly often in the field in the changeable climatic conditions prevailing in the Netherlands.

In a very severe drought the decline in the production of dry matter is not so strong as with a crop which originally has grown with a more ample water supply. In the first period there is, even absolutely, a considerably greater production of dry matter. The crop grown originally with a more ample supply of water is thus much more susceptible to drought. To make this more clear in Fig. 8 the production of dry matter in two periods is again plotted against the supply of water. On the left the production is expressed in grams and on the right in percentages of the dry matter present at the beginning of the periods. With different supplies of water the production of the crop, originally grown with limited water supply and, as a result backward in development, is generally not less and in some cases is even greater than that of the crop originally grown with a more ample water supply. Measured in percentages the growth in the first case has, under all circumstances, been stronger, especially with the young crop. This agrees with the results of Schmid [31]), who also found that the assimilation (production of matter/leaf surface) measured over a long range does not depend on the water supply.

As Singh and Singh [36]) have stated that the production of dry matter in relation to the quantity of water transpired would be subject to large fluctuations, and that the production with a young crop would show a marked minimum, this ratio has been determined for various periods. Table IV gives the quantity of dry matter formed per kilogram of transpired water. The result found by the above mentioned authors is confirmed. In the first period from 10 May–5 June considerably less matter has been formed. It must however be borne in mind that in this period the transpired quantity was found by extrapolation, taking into account the temperature. A lower ratio is repeated in the final stage. Between these, higher and nearly constant values are found. The results agree with those of von Seelhorst [33]), from which it can be derived that grass in a young stage needs comparatively more water than grass in an older stage (see Stålfelt [38])).

From Table IV it appears once more that the production per quantity of water transpired is greater on "drier" soil. The pre-treat-

ment under "dry" conditions has also yielded higher values (see the average values for 12–30 June).

TABLE IV

Quantities of air-dried matter produced per kg of transpired water with various supplies of water

| Original treatment | Water supply | 10/5 5/6 | 5/6 12/6 | 12/6 19/6 | 19/6 30/6 | 30/6 12/7 | 12/7 5/8 | av. 12/6 30/6 | av. 5/6–12/7 | av. 10/5 5/8 |
|---|---|---|---|---|---|---|---|---|---|---|
| "wet" | "very wet" | | 3.2 | 3.0 | 3.2 | 3.6 | | 3.1 | 3.25 | |
| | "wet" | 2.2 | 3.8 | 3.4 | 3.2 | 3.9 | 2.4 | 3.3 | 3.6 | **2.8** |
| | "dry" | | 4.0 | 3.9 | 3.7 | 4.1 | | 3.8 | 3.95 | |
| | "very dry" | | (5.0) | (3.2) | (5.1) | (4.7) | | (4.2) | (4.5) | |
| | "average very wet"-"wet"-"dry" | | **3.7** | **3.4** | **3.9** | | | **3.4** | **3.6** | |
| "dry" | "very wet" | | | 3.9 | 3.1 | | | 3.5 | | |
| | "wet" | | | 3.8 | 3.4 | | | 3.6 | | **3.3** |
| | "dry" | | | 4.3 | 4.1 | | | 4.2 | | |
| | "very dry" | | | (7.2) | (4.7) | | | (6.0) | | |
| | average "very wet"-"wet"-"dry" | | | **4.0** | **3.5** | | | **3.8** | | |

The values obtained on "very dry" soil are placed between brackets in the table, because they are considered to be less accurate. Due to different intensities of transpiration it has not taken the same time before a similar condition has set in, so that the length of the drought in the various experiments has not always been the same. Besides, it may be that the production of matter at the gradual drying of the soil has diminished less than the transpiration (cf. Heinicke and Childers[16]), Schneider and Childers[32])).

It is stated above that the quantity of dry matter formed, calculated over the whole period of growth, amounts to 2.8 g kg of transpired water. That this is lower than the amount which has been found during the strongest growth, must be attributed to the smaller production in the initial and final periods of development.

*The influence of temperature on the production of dry matter.* With the rapid rise of the transpiration rate at high temperature, exhaustion of the supply of water and drying of the crop will occur. It is to be expected that the smaller ability to produce dry matter will, in this case, be especially harmful to the crop, because a higher temperature will by itself be a very favourable growth factor provided that a sufficient supply of water is available. That the latter is really the

case appears from Fig. 9 in which the production of dry matter, divided by the transpiration rate (the transpiration at 15° is taken as the measure of the extent of the assimilating surface), is plotted against the average daily temperature of the periods in which the production is measured. Although the small number of observations does not allow any exact conclusion, yet it is clear that the production of dry matter is to a great degree dependent on the temperature.

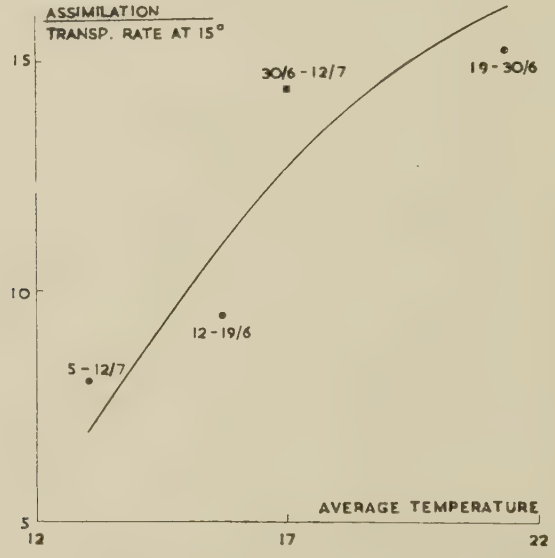


Fig. 9. Ratio of the production of dry matter in 4 periods and the transpiring power (transpiration rate at 15° = 10) in relation to the average daily temperature in those periods.

*The influence of the water supply on the growth in length of leaf and stem.* One of the objects of the investigation was to discover whether it would be possible, from any changes appearing in the growth of the crop, to obtain an indication of the water supply in the soil. If so measurements of the growth of a crop on the field, which are comparatively easy to take, would provide more or less exact information. We have, however, not succeeded in this; the results of the measurements, on the contrary, indicate that the growth in length of leaf and stem are hardly suitable for giving information about the water condition of the soil.

The growth of the leaf of 4 normal primary shoots of each treat-

ment was measured daily by a simple scale; the total growth of 8 shoots being measured. At most 2 leaves of a shoot of the stem are growing at the same time. Only the growth of that leaf which was growing most quickly has been taken for the calculation of the average growth of the leaves. Similarly the growth of the stems has been averaged.

By growth of the leaf is understood the growth of the lamina; by growth of the stem the growth of the sheaths measured from the base to the ligule of the uppermost leaf, and of the true stem measured to the base of the panicle.

With a constant supply of water the growth in length of the leaf on "wet" soil seems to be more vigorous than on "dry" soil and a longer leaf results. The only measurements which provide any insight into this were taken in the period 13-19 June (Fig. 10). For

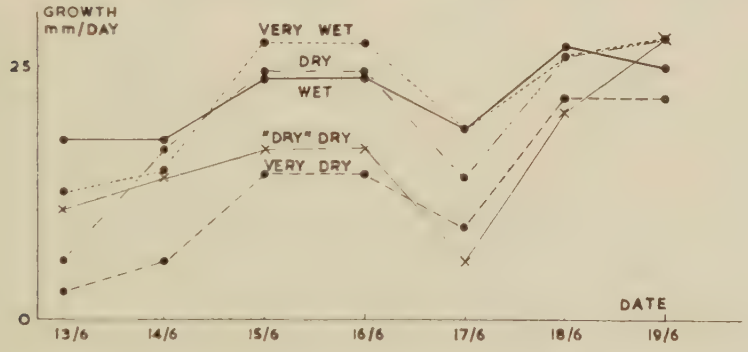


Fig. 10. Growth in length of the leaves of crops with different water supply grown previously on "wet" soil (dots) and growth on a constantly "dry" soil (crosses).

comparison with the measurements of the growth of the crop grown originally on "wet" soil, the growth of the leaf on constant "dry" soil is also given in this figure. The growth of this leaf is considerably smaller. The same is true for the plants also grown on "dry" soil but afterwards supplied with different amounts of water (not represented). This result is remarkable, because the production of dry matter of this latter according to Fig. 7 in this period was, even measured absolutely, larger than that of the growth of the "wet" series treated in a similar way. The growth in length of the leaf thus gives no indication about the production of dry matter. The same

appears to be the case when comparing the growth under various supplies of water to the crops treated originally in the same way. Although according to Fig. 7 the production under the "very dry" conditions has strongly decreased, the growth in length has strongly diminished only in the first few days of the experiment, while there soon appeared a distinct recovery. The other treatments did not give important differences. It appears that the youngest leaves are the best protected against the harmful effects of drought, and that the growth of these, in spite of the dying off of older foliage, has suffered comparatively little damage. Though the water supply remains insufficient, the recovery in the growth of the leaf is also clearly apparent in Fig. 11. At first the growth of the leaf as a result of serious

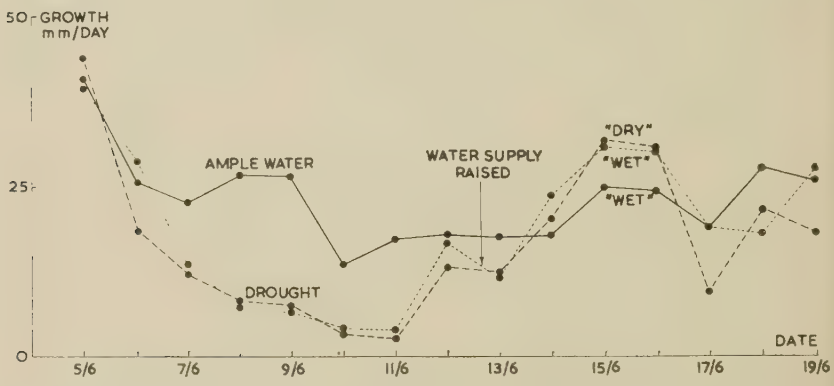


Fig. 11. Growth in length of the leaves of crops grown on constantly "wet" soil (full line) and after setting in of a strong drought (broken lines). The arrow indicates that in one case the previous ample water supply has been restored.

damage by drought was strongly retarded. After 6 days, however, a strong recovery set in. From 12 June a normal quantity of water was again added to one of the series of "dry" pots. The remarkable result of this was that the recovery in both cases proceeded in the same way, and the growth in length of the crop, grown with ample water supply, was for a few days exceeded. In spite of the very insufficient water supply an abnormally vigorous growth has occurred.

Corresponding results have been obtained by measuring the growth of the sheaths before the emergence of the panicles. From Fig. 12 it appears that the growth in length in 4 pots exposed in the same way to intense drought during the first days lagged behind the growth of

plants with ample water supply. Afterwards in this "very dry" series an unmistakable quicker growth has occurred, without improving the supply of water. Ample water was again supplied normally to the "very dry" series from 27 June at intervals of a week. In the series which, on this date, i.e. exactly at the beginning of heading, received again ample water, the growth in length of the stem was

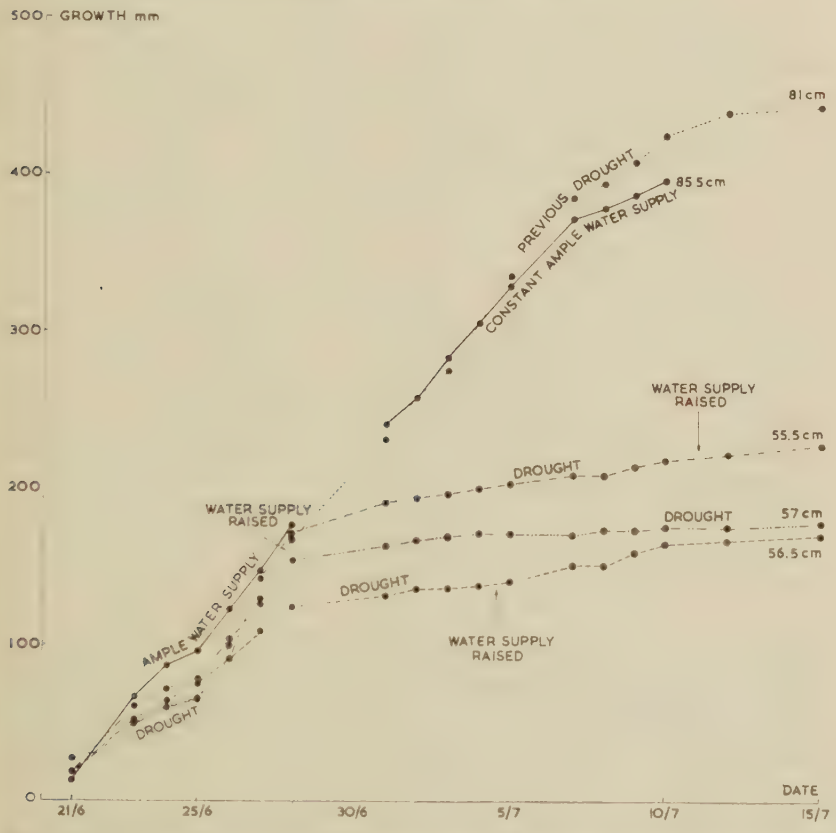


Fig. 12. Growth in length of the sheaths and of the stem, shortly before and after the emergence of the panicles, with a constantly ample water supply (full lines) and in a temporarily strong drouhgt (broken lines). Arrows indicate that the previous ample water supply has been restored. Figures at right side give final length of the crop.

quite normal. The height finally reached was only a little less than that of the normal crop. A period of drought just before the emergence of the panicles has thus only a slight effect on the final height of

the crop, if before the beginning of heading sufficient water is again available.

The continuation of the drought is, on the contrary, highly detrimental to the growth of the stem. In spite of the preceding recovery of the growth of the sheaths, the growth of the stem remained very slow. A recovery of the ample supply of water a few days after heading could not prevent this and has had hardly any influence on growth. This crop and the two others, which one and two weeks later, respectively, received again ample water, all remained 30 cm lower than the normal. The period of the emergence of the panicles appeared thus to be a critical stage at which the further growth of the stem is determined. Von Seelhorst [34]) has shown that the water supply during this stage is essential for the further growth of the stem. The same was found by Moliboga (see Maximow [28])).

The susceptibility of the growth of the stem to changes in water supply appeared also from the measurements taken of the crops originally grown with ample supply of water but for which, from 30 June, the addition of water has been altered. With the "very dry" and the "extremely dry" pots the growth continued for a few days less rapidly and afterwards came entirely to a standstill. With "dry" soil and with a comparatively sufficient supply of water (see Fig. 7), the daily growth in length until 10 July was not more than half of the growth with a more ample supply of water.

*The injurious influence of periods of drought on the yield.* The damage which the yield of straw and grain has suffered from periods of serious drought of various durations (1, 2, 3 and 4 weeks) and beginning at various periods of development of the plants, is given in Fig. 13 and 14.

In both figures the full lines give the decrease in yield in relation to the length of the period of drought. The beginning of these periods follows with intervals of two weeks. The broken lines on the contrary show the change which results from periods of drought of the same duration but beginning at different dates.

Although the damage considered from appearance seemed to be most serious in the first few days after the start of the drought and in this period the rapid dying off of the leaves occurred, the duration of the drought appeared also to have a marked effect. The damage

caused by a period of drought of short duration may thus remain limited to a comparatively slight injury to the yield of the crop.

The results obtained for the straw and grain yield are somewhat different. A drought occurring comparatively early has had a greater injurious influence on the yield of straw, and a drought beginning a

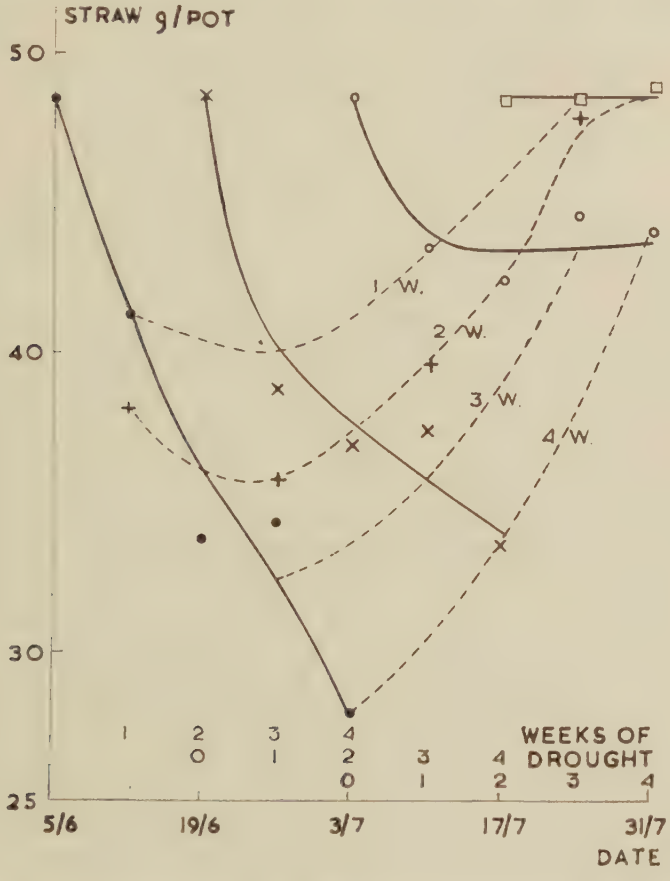


Fig. 13. Damage suffered in the yield of straw as a result of periods of drought, setting in at various times, of 1, 2, 3 and 4 weeks, respectively. Full lines give the influence of the length of the periods of drought, broken lines the influence of periods of drought of the same duration setting in at different times. The dates at the abscissa have reference to the end of the periods of drought.

little later on the yield of grain. Towards the end of the development a drought had still an unfavourable influence on the grain, but not

on the yield of straw. The same result was obtained by Bünger [8]).

The most serious damage to the yield of grain occurred when the drought prevailed in the period just before or during the emergence of the panicles. This result is also in agreement with those of former

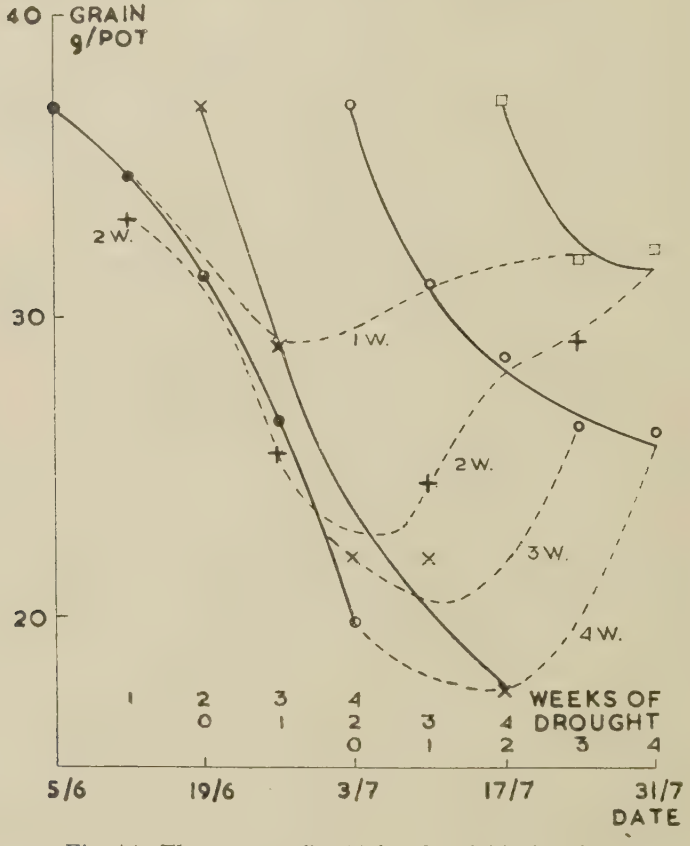


Fig. 14. The same as fig. 13 for the yield of grain.

investigations (von Seelhorst [34]), Brounov, Pullmann, Moliboga, cf. Maximow [28])).

From the foregoing it appears that the ratio between the yields of straw and grain varies according to the stages of development of the crop in which the drought has occurred. Also the crops grown constantly with various water supplies show a different ratio; viz. 1.28 with the comparatively "wet" soil and 1.02 with the comparatively

"dry" soil. In the latter case a considerably greater part of grain has been formed. An extreme drought occurring early had a similar influence; a drought setting in later, however, resulted in a much more unfavourable ratio. As an example the following figures give the ratios of straw and grain found in crops exposed to periods of drought occurring at various stages of development.

| Period of drought | Ratio Straw/Grain |
|---|---|
| 29/5–12/6 | 1.14 |
| 5/6–19/6 | 1.08 |
| 12/6–26/6 | 1.41 |
| 19/6– 3/7 | 1.69 |
| 26/6–10/7 | 1.62 |
| 3/7–17/7 | 1.48 |
| 10/7–24/7 | 1.66 |
| 17/7–31/7 | 1.55 |

These results also are in agreement with those of Bünger [8]), who likewise found that a drought which sets in early has a favourable influence and one occurring late has an unfavourable influence on the ratio.

A change in the ratio can occur (v. Seelhorst [34])) when the weather conditions towards the end of the development bring out tillers which do not ripen. In the dry summer of 1947, however, this phenomenon has not appeared.

*The influence of periods of drought on the composition of the crop.* The counting of the number of panicles and the determination of the average weight of the grains enabled a further analysis of the yield of grain to be made.

In Fig. 15 a survey is given of the influence, which the periods of drought occurring at various times have had on the yield of grain. Here the yield, the number of panicles, the weight of the grains and the number of grains per panicle for the crop on constantly "wet" soil is taken as 100, and the results of the other treatments are given in percentages.

The figure shows the influence of periods of two weeks of drought on the yield, which has already been given in Fig. 13. The earliest period has not seriously reduced the number of panicles. Against a slight reduction of the number of grains per panicle there is a slight increase in the weight of the grains. The periods of drought following

this have strongly reduced the number of panicles. In the period 19 June–3 July all secondary shoots have died off. This is largely compensated by a greater number of grains per panicle, because only the stronger primary panicles have survived. After the second period of drought, however, the number of grains has also been reduced. At the same time the weight of the grains appears to decrease regularly, with one exception to be discussed below.

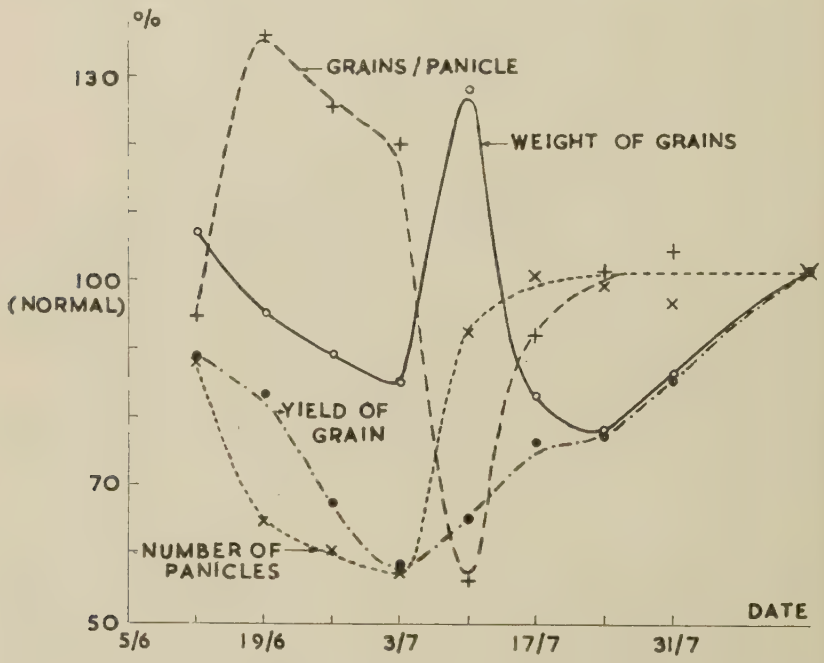


Fig. 15. Influence of periods of drought of 2 weeks occurring at various dates on the magnitude and the composition of the yields of grain, expressed in percentages of the results obtained with constantly ample water supply.

Drought occurring after the emergence of the panicles has had no further influence on the number of panicles which came to ripeness. A drought during the period 26 June–10 July in which the panicles emerged, was disasterous for the forming of the grains. This crop was characterized by a large number of barren ears. As a result of this reduction of the number, however, very heavy grains have developed. Drought which set in later has only influenced the weight of the grains unfavourably. When the injurious influence came still

later this injury has become proportionately smaller (c.f. Büng e r [8]).

**Summary**

The transpiration rate of oats grown in Mitscherlich pots with a sandy soil appeared to increase with the forming of the leaf, and to reach a very early maximum before the growth of the leaves ended, as a result of the dying off of older leaves and part of the secondary shoots. After this the transpiration rate gradually diminished, although towards the end of growth it fell more quickly.

Relative humidity appeared to influence the daily transpiration only at relatively low temperatures when the speed of the process is slow. At higher temperatures the transpiration rate became independent of air humidity. Under these circumstances temperature proved to be the limiting factor, $Q_{10}$ being of the order of 2–2¼ in the range of 15–25°. It is probable that a higher temperature lowers the resistance of the root system to the absorption of water.

A positive influence of light intensity on the transpiration rate was observed at low temperatures only. At moderate temperatures no variation in the rate has been observed with different cloudiness of the sky. An indication has, however, been obtained that a clear sky decreases the transpiration rate on very hot days.

No influence of wind velocity on the daily transpiration could be detected.

The ratio of the transpiration of plants grown with limited water supply to that of plants grown with ample water agrees approximately with the ratio of dry or fresh matter. It has not, however, been concluded from this that the magnitude of the transpiration is determined by the extent of the transpiring surface, as it is more probable that the ability of the roots to absorb water was limiting the transpiration in the present experiments. From this and other facts it became obvious that an adaptation of the root system to a limited water supply occurred. In the long run the plant becomes rather independent of the water content of the soil.

At low temperature the transpiration rate of plants grown on "dry" soil on a dry weight or fresh weight basis is even larger than that of plants grown with ample water. At higher temperature the transpiration rate is less in the first case, indicating that the supply of water becomes a limiting factor.

The transpiration rate appears to be very dependent on a change of the water supply. The transpiration rate of a crop originally grown on "dry" soil exceeds, under similar conditions, that of a crop originally grown on "wet" soil. With a lower water supply the rate diminished rather strongly, sometimes with a simultaneous wilting followed, however, after a few days by a recovery. With an increasing water supply the transpiration rate, after a few days, increases more strongly than at the beginning of the experiment. Presumably the explanation of these facts is to be sought in the adaptation of the roots.

The damage caused by drought is worse in proportion to whether the crop has originally had a more ample supply of water.

The crop grown with constantly limited water supply forms, after some time, relatively (per dry matter already formed) more matter than the crop growing constantly with ample water, so that the ratio of the dry matter becomes gradually more favourable and the difference in the yield of grain is relatively small.

With an alteration in the water supply the assimilation appears to be determined by the amount of water added. The production of the crop grown originally on "dry" soil markedly exceeds under similar conditions relatively (per unit of dry matter) the growth of the crop originally grown on "wet" soil and sometimes even absolutely. With a later ample supply of water arrears can largely be nullified.

With a drought during the beginning of the forming of the grain all the dry matter formed comes to grain (premature ripening), so that the difference in grain production with plants provided with ample water is temporarily very small.

With an insufficient water supply more dry matter is formed, per quantity of water transpired, than with an ample water supply. With a crop grown previously on "dry" soil this ratio, under comparable water conditions, remains greater than with a crop previously grown on "wet" soil. The quantity of dry matter formed per quantity of water transpired is the highest in the middle of the period of growth, and for some considerable time remains fairly constant. With an ample supply of water this amounts, for example, on the average to 3.6 g air-dried matter per kg of water. At the beginning and at the end of the period of growth this ratio is less favourable (calculated for the whole period at 2.8 g air-dried matter/kg water).

Temperature has approximately the same effect on the production of dry matter as on the transpiration rate. If, at a high temperature, there is a deficiency of water, this will not only cause serious damage, but it will also have the result that the crop will not profit by the otherwise favourable conditions.

The length of the leaf remains smaller on constantly "dry" soil than on constantly "wet" soil. When the water supply has been changed the growth in length of the leaves of the crop previously grown with limited water supply remains smaller than those from a crop previously grown with ample water supply, though the production of dry matter may differ little in both cases.

The growth in length of the leaf is comparatively little influenced by the water supply. Even with very serious damage by drought the growth generally does not stop. After a few days the growth under these conditions may even be faster than that of plants grown with a constant and ample water supply. The same has been observed with the growth of the sheaths which only becomes temporarily slower.

For the growth of the stem the water supply during the emergence of the panicles is critical. A period of drought just before this stage has, on the

whole, no injurious influence. With a drought during the emergence of the panicles the growth remains very slow and a short crop results. If, some days later, ample water is available, this has no influence on the growth of the stem.

A period of drought has all the more injurious influence on the yield as the duration of this period is lengthened. A drought prevailing during and shortly before the emergence of the panicles has the most injurious influence on the yield of grain, and a somewhat earlier period on the yield of straw. A drought which occurs earlier is less injurious on the yield of grain. A drought which occurs late influences the yield of straw little or not at all, but it does influence the yield of grain.

The ratio between straw and grain with a crop grown on "dry" soil is considerably less than with a crop grown on "wet" soil. A drought beginning early decreases this ratio and one occurring later increases it.

A drought occurring comparatively early reduces the number of secondary shoots, which, however, is compensated by a greater number of grains per panicle. This number becomes again less if the drought occurs later. The weight of the grains also diminishes.

A drought occurring during the emergence of the panicles results, however, in a considerable reduction of the number of grains per panicle by the production of barren ears. This is partly compensated for by considerably heavier grains. A late drought only influences the weight of the grains which remains low.

Received December 17th, 1943.

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# PRESOAKING AND COLD HARDINESS IN MAIZE

by GORDON HASKELL

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*Introduction.* The germination of maize seed after cold room treatment is effected by the external environment. This includes variation between different lines, temperature and medium in which the seeds germinate and time in the cold rooms, together with their various interactions (H a s k e l l[3])). Other factors, such as genetical constitution of the embryo e.g. whether seeds are starchy or sugary, also play a role (H a s k e l l and S i n g l e t o n[4])) but seed shape is not important.

Another variable is moisture content of the seed before treatment. An investigation was therefore made to determine whether moisture content of seeds prior to actual germination influences their resistance to cold germinating conditions.

*Materials and Methods.* The moisture content of seeds of four inbred sweet corn lines were determined by an electric moisture testing outfit. The seeds were all the same age and had been handled alike in the previous autumn, e.g., they had the same drying conditions. Inbreds C40, P51 and T51 were very similar in moisture content but C95 was unusually low, (Table I).

Thirty lots of twenty-five seeds each of these inbreds and a hybrid dent corn, Conn. 520, were placed in test-tubes. Two tubes of each strain were used as controls. Tap water was added to four tubes of each. After twelve hours this was replaced by fresh tap water and water added to an additional four tubes of each strain. This was repeated till the first batch of seeds had been soaking for 60 hours and the last batch for 12 hours. All in all, there were four lots of seed of each line soaked for 0, 12, 24, 32, 48 and 60 hours in water, i.e. a total of six batches.

The seeds from each soaked batch were sown in seed-boxes in rows, the order according to length of time soaked. Thus each seed-box contained one inbred only but with seeds that had received different soakings. Two rows of each were placed side by side. Half the seed-boxes were placed in the 40° F room for 8 days and the other half in the 60° F room. The boxes were watered when required. Later they were removed to a warm greenhouse and the maximum germination recorded. Percentages were transformed to angular values and the mean angles obtained. The average values of the four inbreds were also calculated. These data are given in Table I.

TABLE I

Germination (in mean angles) of Sweet corn seeds pre-soaked before subjecting to cold treatment

| Hours soaked | | C40 | P51 | T51 | C95 | Sweet corn Mean | Conn. 520 |
|---|---|---|---|---|---|---|---|
| 0 | 60°F | 59.4 | 57.1 | 49.8 | 45.0 | 52.8 | 62.1 |
| | 40°F | 45.0 | 58.2 | 43.9 | 45.0 | 48.0 | 48.5 |
| 12 | 60°F | 64.9 | 67.2 | 67.2 | 36.8 | 59.0 | 59.5 |
| | 40°F | 47.4 | 50.9 | 48.7 | 29.3 | 44.1 | 41.4 |
| 24 | 60°F | 61.0 | 57.1 | 60.8 | 26.1 | 51.3 | 67.2 |
| | 40°F | 54.4 | 54.6 | 45.0 | 31.8 | 46.5 | 39.0 |
| 36 | 60°F | 66.4 | 52.0 | 52.0 | 30.5 | 50.2 | 63.6 |
| | 40°F | 41.5 | 52.1 | 47.4 | 26.5 | 41.9 | 53.2 |
| 48 | 60°F | 62.3 | 47.4 | 68.5 | 28.0 | 51.6 | 65.2 |
| | 40°F | 59.5 | 45.0 | 47.4 | 24.8 | 44.2 | 55.6 |
| 60 | 60°F | 33.1 | 43.9 | 68.1 | 31.8 | 44.2 | |
| | 40°F | 40.1 | 48.7 | 41.5 | 28.0 | 39.6 | |
| % Moisture content of original seed | | 8.36 | 8.10 | 7.32 | 4.82 | | — |

*Discussion.* The germinations from the 40° F treatments were much lower than those for 60° F. This suggests that damage by soil pathogens was not so heavy as at 50° F. (Haskell[2]). Even so, mean germination of the inbreds at 60° F was only 52.8°, so that there was still a fair amount of damage at this temperature.

From the results at 40° F, it seems there was a gradual effect from pre-soaking on germination after cold treatment. However the results are rather irregular and the decrease only slight.

The analysis of variance of the data is given in Table II. $\chi^2$ values were obtained, using $V_0 = 820.7\,25$ (Mather[5]). Values are obtained directly using this as divisor of the corrected S.S. Main

TABLE II

Analysis of variance of Sweet corn data presented in Table I

| | S.S. | N | $\chi^2$ | P | M.S. | V.R. | t | P |
|---|---|---|---|---|---|---|---|---|
| Varieties V . . . . . | 3999.232 | 3 | 121.824 | < 0.001 | 1333.077 | 15.117 | | <.001 |
| Temperature T . . . | 672.752 | 1 | 20.493 | < 0.001 | 672.752 | | 2.76 | 0.02–0.01 |
| Soakings S . . . . . | 479.421 | 5 | 14.604 | 0.02–0.01 | 95.884 | 1.087 | | Not sig. |
| V–T . . . . . | 365.229 | 3 | 11.126 | 0.01 | } 88.183 | | | |
| V–S . . . . . | 1222.067 | 15 | 37.226 | 0.001 | | | | |
| T–S . . . . . | 157.885 | 5 | 4.809 | 0.5 | | | | |
| V–T–S . . . . . | 537.250 | 15 | 16.366 | 0.3–0.5 | | | | |
| Total . . . . . | 7433.835 | 47 | | | | | | |

$V\varnothing = 820.7/25 = 32.828.$

effects due to varieties and temperatures give very low probabilities, and for pre-soaking $P = .02—.01$, so that all are highly significant. Varieties-soaking interaction gives highly significant values ($P = = .001$) indicating that inbreds react differently according to their soakings. Varieties-temperatures also give a low probability, but the temperature-soakings reaction is not significant.

In view of the highly significant values for varieties-temperatures, varieties-soakings, the S.S. of these were pooled for 18 df. and the mean square so obtained. This is used as error mean square to test more precisely whether soaking has any effect on cold resistance. The variance ratios then give highly significant values for strain differences and temperature effects but not for the influence of pre-soakings. It thus appears that pre-soaking sweet corn seeds does not alter their reaction to cold treatment; the reaction of individual varieties is of greater importance.

It is possible that pre-soaking has some effect on the eventual germination in producing and hastening plumule emergence after transference to warmth (Andrews and Beals [1]). Pre-soaking might, therefore, be of some use in speeding up seedling growth. This would allow quicker potting on of seedlings selected for ability to withstand low temperatures where cold rooms are used as substitutes for field sowings.

**Summary**

Seed of four inbred sweet corns and one dent hybrid corn were pre-soaked in tap water prior to receiving 60°F and 40°F treatment. Pre-soaking did not alter their behaviour to cold treatment, although on transference to the warmth, germination was slightly more rapid after pre-soaking.

Received December 20, 1948.

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# MANGANESE IN RELATION TO PHOTOSYNTHESIS

## I CARBON DIOXIDE ASSIMILATION AND THE TYPICAL SYMPTOMS OF MANGANESE DEFICIENCY OF OATS *)

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*Introduction.* The fact, that extreme manganese deficiency often is accompanied by chlorosis, originally led to the assumption, that manganese was important for the formation of chlorophyll. According to Bishop[1]) the absence of manganese is reflected in the inability of the plant to synthesize chlorophyll. McHargue[9]) concluded that "manganese plays a role of importance in the synthesis of chlorophyll".

The interesting experiments of Somers and Shive[15]) however prove that the dry weight and the condition of the plants is related to the Fe–Mn ratio and not to the absolute concentrations of these nutrients. Chlorosis in the case of manganese deficiency is due to iron toxicity.

There is little difference of opinion on the fact that plants poor in manganese show retarded growth of leaves, stems and roots, whereas the starch and sugar content of the leaves is markedly reduced. (McHargue)[10]), Ethel Eltinge[4]).

Emerson and Lewis[5]) studying the photosynthetic efficiency of *Chlorella pyrenoidosa* mention the fact that in their experiments shortage of micro elements in the culture medium results in diminished photosynthetic activity, whereas manganese plays a major part in maintaining or restoring the maximum value, often within a short period after it is supplied.

*) Investigations on the influence of manganese on photosynthetic processes were carried out by the author just before and during the beginning of the war. Owing to changing circumstances this work could not be continued and consequently remained fragmentary in some aspects. Those results, which still seem to be of sufficient importance will be published in a series of papers, of which this is the first.

When plant growth is retarded by the absence of some essential element, reduced photosynthesis is not necessarily the prime cause of the lower yields; on the ground of the experiments of Burström [2] on the necessity of manganese for nitrate assimilation, the assumption that a disturbed nitrogen balance might be the cause of retarded growth and of chlorosis is not a priori to be rejected. Gassner and Goeze [7] showed that the photosynthetic activity of the leaves of wheat plants, deficient in potassium, was decidedly higher than that of plants with normal potassium content.

As positive proof of reduced photosynthetic activity in manganese-deficient higher plants is still lacking, it has been the author's object to compare carbon dioxide assimilation in manganese deficient leaves of *Avena sativa* with that in normal leaves under controlled circumstances and to correlate the results with the symptoms of the disease.

*Methods.* The general layout of the apparatus necessary for the measurement of carbon dioxide assimilation consists of an assimilation chamber, suspended in a constant temperature waterbath, a device to measure the quantity of air that passes through the assimilation chamber, an absorption vessel to determine the residual carbon dioxyde and a light source of adequate intensity and sufficient constancy. The heat rays of the light must be absorbed by waterfilters (Fig. 1).

The assimilation chamber was made of two plate glass windows and three side strips, joined with de Khotinsky cement; the inside dimensions were 100 × 235 × 10 mm. In the glass cover were inserted a long gas inlet tube and a short one as gas outlet. The cover was fixed on top of the assimilation chamber with a mixture of resin and wax. The bases of the leaves were put into a small container, filled with water, so that evaporation could proceed undisturbed; the leaf bases were fixed and the top of the container was closed with plasticine. At the end of the experiment the leaves were cut just above the plasticine and put on a 13 × 18 photographic paper, together with a piece of millimetre paper of exactly 11 × 5 cm and pressed flat under a glass plate. The photographic paper was illuminated and an accurate print of the surface of the leaves was obtained, which was measured with the aid of a plani-

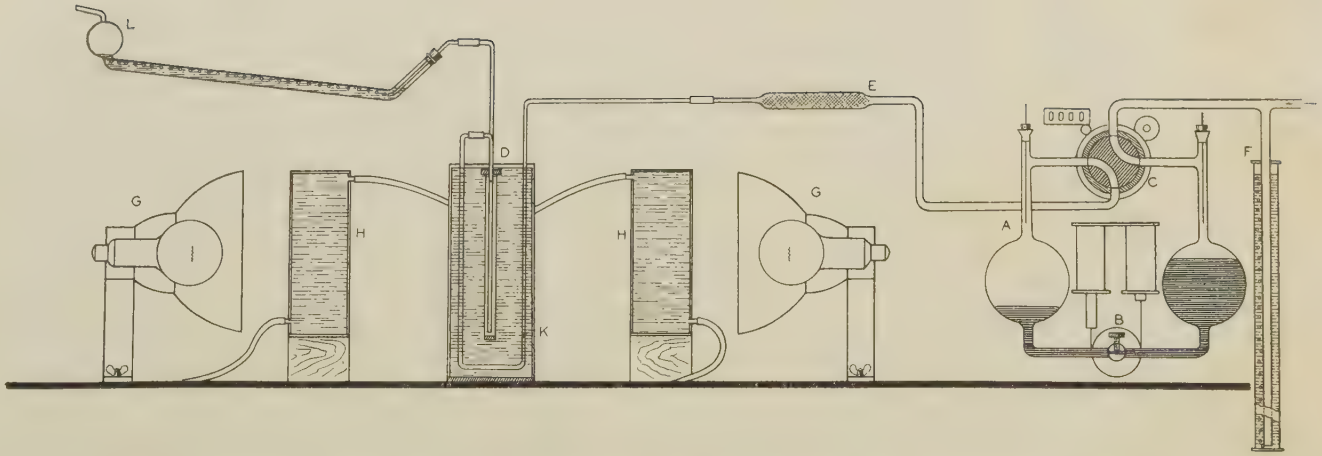


Fig. 1. Schematic layout of the apparatus for the determination of carbon dioxide assimilation.

*A*. Mercury pump with electromagnetic stopcock B, reversing valve C and pressure regulating waterlock F.
*G*. 200 watt tungram lamps with reflectors.
*H*. waterfilters for heat absorption.
*K*. constant temperature water bath.
*D*. assimilation chamber.
*L*. Pettenkofer absorption tube filled with $Ba(OH)_2$ sol.

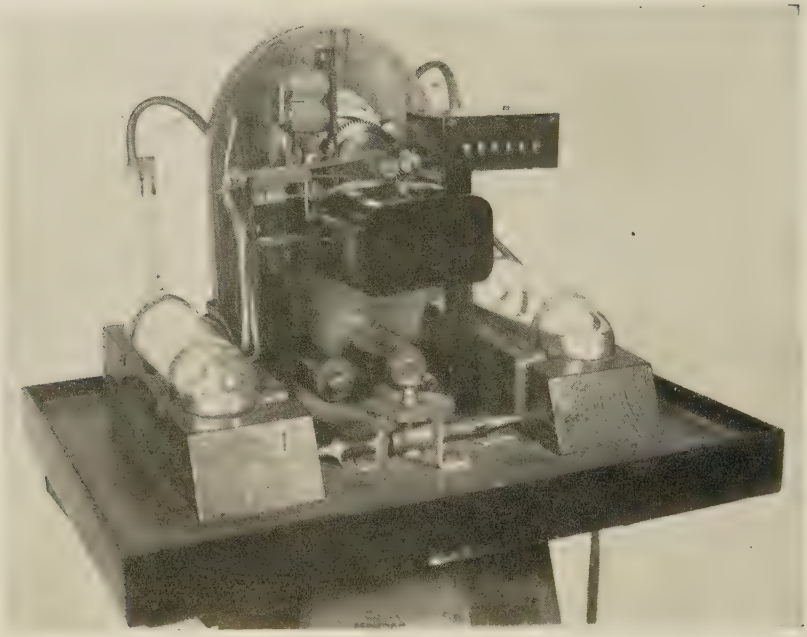

Fig. 2. Mercury pump, with small encased sweeper motor reversing valve with cog wheel, electromagnetic stopcock with regulator screw, starter electromagnet, and counter.

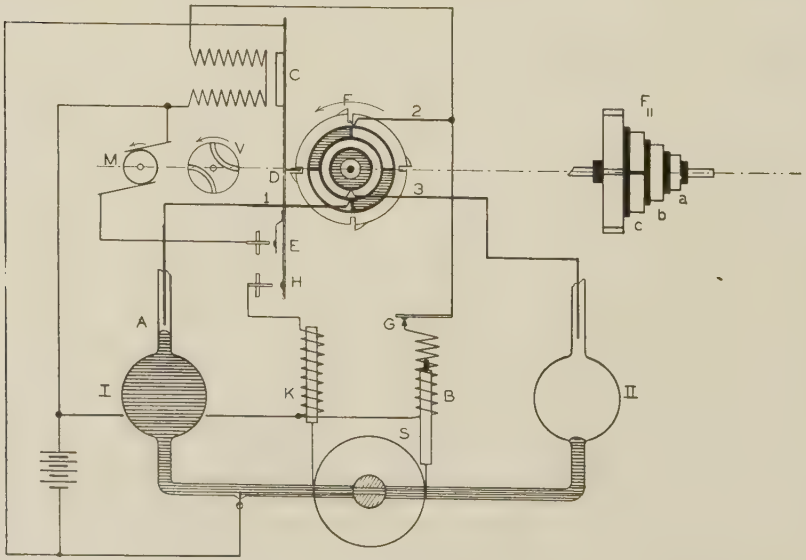


Fig. 3. The electric circuit of the mercury pump.
M. electric motor, with reducing gear connected to reversing valve (V) and rotary switch F.
$F_{II}$ side view of rotary switch, which consists of 2 isolated copper rings a and b each connected to two segments of the quadripartite current-reversing contactring c.
D. mechanical device to ensure a periodical rotation of 90° of the reversing valve.
S. electromagnetic stopcock.

meter. To correct for eventual shrinkage of the paper, the surface $A$ of the millimetrepaper was measured as well, which yielded the correction factor $55/A$.

The air was taken from an air bomb and measured by means of the device, outlined in figs. 1 and 2. This consisted of two glass vessels with a capacity of 250 cc each, one of which was filled with mercury. By means of a rotating stopcock $C$ one of the glassvessels could be connected with the air bomb, whereas the other one was connected with the assimilation chamber $D$. At the moment when the first vessel was filled with air and the other one with mercury, an electrical contact was made at $A$; this closed the electromagnetic valve $B$, which connected the two glass vessels, for an instant, and a small electromotor (sweeper motor) turned the stopcock 90°. Just before the end of the cycle the electromagnetic valve was opened again and a new cycle could begin. The number of turns was counted by means of a small counter.

To assure a constant pressure of the air in the vessels, the air supply tube was provided with a side tube, which plunged into a water vat with a constant level $F$.

The velocity with which the mercury flows from one vessel into the other controls the frequency of the strokes and the quantity of air per hour. This could be regulated with a screw $B$, which widens or narrows the canal in the electromagnetic stopcock.

To avoid possible damage to the leaves by mercury vapor, an adsorption tube $E$ containing gold leaves was inserted in the air supply line.

The air which left the assimilation chamber passed through a Pettenkofer absorption tube $L$, filled with barium hydroxide solution. To ensure a prolonged contact of the air with the solution 2–3 drops of a saponine solution (10%) were added, which raised the surface tension and stabilized the airbubbles for some time. The whole apparatus was placed in a room of constant temperature (25°C).

*Operation of the electric circuit,* (Fig. 3). At the moment the glass vessel I is filled with mercury, contact is made at $A$ and the current passes from the contact spring nr. 1 through the revolving commutator to nr. 2, activating the electromagnet $B$ of the mercury stopcock, which closes the connection between the two vessels. The descending iron core disconnects the circuit of the electromagnet at $G$. At the same time the relay $C$ is activated, pin $D$

is lifted from the notch in the rotary switch $F$ and the motor contact $E$ is closed. The motor $M$ turns the two way valve $V$ and the rotary switch, reversing the connections between the air inlet and outlet tubes and the glass vessels.

Just before the end of the cycle the stud at $F$ lifts the pin $D$ which closes the contact $H$ energizing the left electromagnet $K$ of the mercury stopcock. As the mercury flows into the vessel II, the contact at $A$ is opened, the relay is released, the motor stops, the apparatus is ready for the next cycle. Contact spring Nr. 3 is now connected to Nr. 2.

*Analytical procedures.* In the Pettenkofer absorption tubes the air was first replaced by $CO_2$ free air and then they were filled with 100 cc barium hydroxyde solution, equalling 100 cc oxalic acid, corresponding to 50 cc $CO_2$ at 760 mm Hg pressure and 0°C.

After the experiment the contents were titrated with the necessary precautions to exclude the air. To determine the $CO_2$ percentage in the air from the bomb, the apparatus is set to work and stopped after 50 or 100 strokes; the total quantity of $CO_2$ divided by 50 or 100 gives the amount of $CO_2$ per stroke. In order to be able to correct for changes in barometric pressure and temperature, both values have to be noted during the experiments.

The fact, that the contact which stops and reverses the flow of the air is made in a tube of small diameter as compared to the diameter of the air containers, assures an uniform and very accurate dosage of the air.

The volume of the air containers was determined by weighing the quantity of water which was displaced in a receiver by the air from a noted number of strokes from the apparatus.

*Plant material.* The oat plants were grown on healthy soils as well as on manganese-deficient soils of different origin. Nutrient solutions with and without manganese were also used. It proved to be better not to use plant material with pronounced symptoms of extreme manganese deficiency; in most cases the third and fourth leaves were taken for the experiments, which did not differ much from healthy leaves, save for growth retardation.

*Results.* In the following table the results from the first experiments are summarized.

Though there is a wide variation in the assimilative power of the control plants of different origin, carbon dioxyde assimilation

TABLE I

| Nr. Exp. and date | plant material | duration experiment | number of strokes of gasometer | total vol. in *l* of 0°C and 760 mm Hg | total cc $CO_2$ (0° 760 mm) in this vol. | cc $CO_2$ (0° 760 mm) assimilated | total leaf surface $cm^2$ | mg $CO_2$ assimilated by 100 $cm^2$ in 1 hour | decrease of assimilation in % of corresp. control |
|---|---|---|---|---|---|---|---|---|---|
| 1*a*. 24/2 | +Mn | 45'40" | 60 | 12.95 | 6.54 | 3.84 | 118.3 | 6.41 | |
| 1*b*. | 0Mn | 45' | 88 | 20.22 | 10.21 | 2.06 | 82.8 | 4.92 | 23.3 |
| 2*a*. 5/3 | +Mn | 45' | 85 | 19.64 | 9.92 | 4.95 | 80.3 | 12.18 | |
| 2*b*. | 0Mn | 45' | 88.5 | 20.34 | 10.27 | 3.95 | 71.5 | 10.91 | 10.4 |
| 3*a*. 4/4 | +Mn | 45'30" | 85 | 19.82 | 10.00 | 3.97 | 105.1 | 7.46 | |
| 3*b*. | 0Mn | 45' | 91 | 21.03 | 10.62 | 1.89 | 102.8 | 3.65 | 51.2 |

Nrs. 1*a* and 1*b*, plants cultivated in glass-house.

Nr. 1*a*. healthy soil, Nr. 1b. manganese-deficient soil.

Nrs. 2*a*, 2*b*, 3*a* and 3*b*, plants cultivated in watercultures, with and without $MnSO_4$.

In each case the plants + and 0Mn belong together and were grown under identical circumstances, save for the manganese supply.

of all the leaves from the manganese-deficient plants is lower than that of the corresponding control plants.

In particular the difference of 51,3% in the case of numbers 3*a* and 3*b* is significant.

As the variation in assimilative power of the healthy leaves might be associated with their manganese content, in the next experiment the manganese content of the leaves was determined. The results of the second experiment are summarized in table II.

TABLE II

| Nr. exp. and date | plant material | duration experiment | number of strokes of gasometer | total vol. in *l* (0° 760 mm) | total cc $CO^2$ (0° 760 mm) in this vol. | cc $CO^2$ assimilated during exp. | total leaf surface $cm^2$ | manganese content mg/kg dry matter | mg $CO_2$ assimilated per 100 $cm^2$ in 1 hour | decrease of assimilation in % of corresp. control. |
|---|---|---|---|---|---|---|---|---|---|---|
| 4*a*. 30 10 | +Mn | 48' | 80 | 18.29 | 9.19 | 5.24 | 133.4 | 76.5 | 9.71 | |
| 4*b*. | 0Mn | 44'11" | 80 | 18.29 | 9.19 | 2.03 | 164.1 | 6.8 | 3.31 | 66.1 |
| 5*a*. 1 11 | +Mn | 45' | 78 | 18.25 | 9.17 | 2.41 | 83.2 | 83.4 | 10.05 | |
| 5*b*. | 0Mn | 45' | 80 | 18.55 | 9.31 | 1.90 | 127.0 | 2.1 | 3.95 | 60.6 |
| 6*a*. 2/11 | +Mn | 45' | 76 | 17.49 | 7.82 | 5.11 | 97.3 | 47.6 | 13.85 | |
| 6*b*. *) | 0Mn | 45'7" | 78 | 17.95 | 9.06 | 1.37 | 104.8 | 1.4 | 3.46 | 75.0 |

*) These leaves were chlorotic.

From the results reported in table II it is apparent that in all the plants which were deficient in manganese, carbon dioxide assimilation is reduced to 25–40% of the normal rate.

The question arises whether this is due to some direct role, that manganese plays in the process of photosynthesis or whether it is simply the result of chlorosis, which often accompanies manganese deficiency. To avoid the latter possibility, special care had been taken to choose leaves, which did not show any signs of pronounced chlorosis; it is the author's experience, that plants, deficient in manganese, often do not show any notable symptoms, except for a retarded growth and smaller yields *).

In experiment 4*b* for example the colour of the leaves did not differ perceptibly from that of the normal leaves; nevertheless the assimilation amounted to only one third of normal. The same was also the case with the other experiments, except for experiment 6*b* where the leaves were chlorotic. In that case the photosynthetic activity was reduced by 75%. Though it is to be expected that chlorosis plays some part in the reduction of the rate of carbon dioxyde assimilation, it is certain that in no case did the chlorophyll content of the leaves in these experiments differ so much from that of the control plants, that a reduction of the photosynthetic activity by 60–75% could be accounted for.

The question may be raised, whether the determination of the chlorophyll content of the leaves might have been of use to eliminate the influence of chlorosis on the process of photosynthesis.

According to the recent investigations of Gabrielsen [6]) the chlorophyll concentration of the fully developed leaves of different plants exceeds by far the optimum value of the chlorophyll factor; there is little difference in the photosynthetic activity of leaves which contain 4–5 mg $(a + b)$ chlorophyll $dm^2$ and those which contain twice as much. When this is the case the determination of the chlorophyll contents of the leaves would have been of little value, especially because care has been taken, not to use any leaves with a pronounced chlorosis.

It seems plausible that in the present experiments reduced photosynthesis is due to lack of manganese and that manganese plays some direct role in the process.

*Discussion.* The fact that manganese deficiency reduces carbon dioxyde assimilation enables us to interpret a number of divergent

*) Rademacher [13]) observed that notwithstanding the so called resistant oat race Weibulls Argus, when growing on manganese deficient soil, only sporadically showed manganese deficiency symptoms, it produced only 55% of a normal yield.

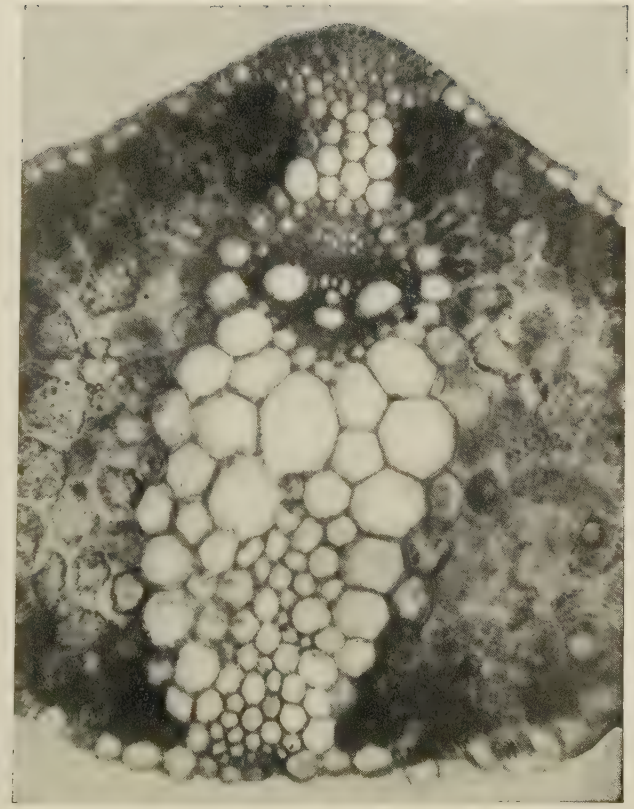

Fig. 4 a. Microphotograph (135x) of a transverse section through the main nerf of a healthy oat leaf (2d leaf from top at 1/4 from the base).

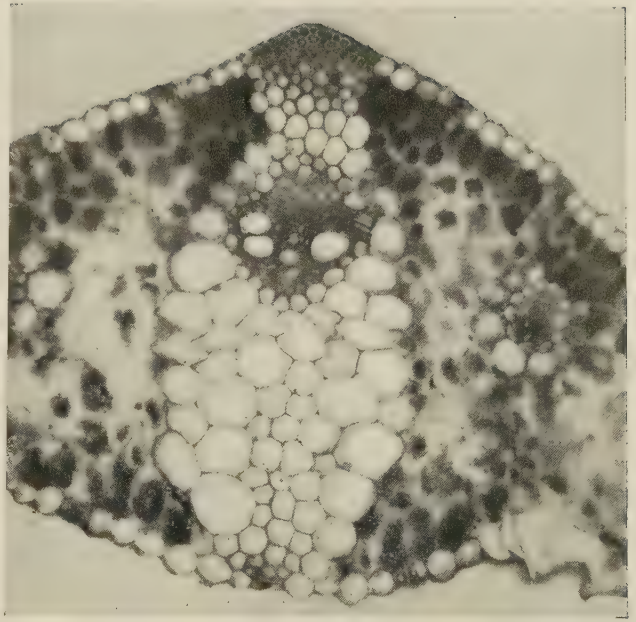

Fig. 4 b. Microphotograph of a transverse section through the main nerve of an oat leaf from a manganese-deficient oatplant of the same age as A. (2d leaf from top at 1/4 from the base). Note the smaller number and size of the sclerenchymacells, the smaller area of the central vascular bundle and the smaller number of reinforced cells in the lower part of the leaf. (135x)

symptoms, characteristic for the disease, from a central point of view.

*The leaves.* The most common effect is a retardation of growth: the plants are shorter, the leaves are smaller, thinner and less rigid and finally necrotic spots appear. The top part of the leaf often drops over, forming a sharp kink; this may occur not only on a place where the tissue is dying [11]), but on intact leaves as well.

According to Mc Hargue [10]) the starch and sugar content of the leaves of oat plants, deficient in manganese, is much smaller than that of healthy leaves. Ethel Eltinge [4]) showed the same for tomato leaves.

In several cases it has been reported that damage to cereals, due to late night frosts was more pronounced in plants deficient in manganese than in normal plants. As frost resistance is closely related to the turgescens of the cells and the latter to the content of sugars in the cellsap, which has been shown to be low in manganese-deficient plants, the relation between frost resistance, manganese deficiency and reduced photosynthesis becomes intelligible.

As a direct result of reduced photosynthesis less assimilates are available for the building up of sugar, starch and cellulose, which explains in a simple manner most of the above mentioned symptoms. By lack of adequate material, the cell walls are thinner and consequently the elasticity of the cell structure, which counteracts the turgor inside the cells is diminished, and this accounts for the slackness of the leaves. As may be seen from the microphotograph fig. 4*a* en *b*, the volume and number of the sclerenchymacells in the manganese-deficient leaves is greatly reduced, resulting in a weakness of the leafribbs, which causes the leaves to drop over with a kink.

TABLE III

| | 0 Mn | +Mn | diff. % |
|---|---|---|---|
| Number of sclerenchymacells reinforced epidermic cells included | 63 | 82 | 30,0 |
| Area of the sclerenchymacells, reinforced epidermal cells included | 0,111 mm² | 0,136 mm² | 22,6 |
| Area of the xylem in the central vascular bundle | 0,102 mm² | 0,154 mm² | 51,0 |
| Thickness of the leaf at the place of the main nerve | 0,59 mm | 0,78 mm | 30,5 |

The microphotographs were taken from sections cut at a quarter of the way up the leaves (the second from the top) of plants of the same age,

which were grown with and without manganese. The number of sclerenchymacells, the reinforced epidermal cells included, of the leaf with manganese, was 30% larger than that of the leaf deficient in manganese. The area of the xylem in the central vascular bundle was 51% larger and the thickness of the leaf at the place of the central rib was 30% greater (Table III).

The question arises, to what extent respiration in the leaves is influenced by manganese deficiency; according to Lundegardh [12]) Mn has a decidedly favourable influence on the fundamental respiration of roots.

In a preliminary experiment the quantity of $CO_2$ produced by respiration was determined in 5 cases directly after illumination.

TABLE IV

| Respiration of leaves of plants grown with and without Mn | | | | | | |
|---|---|---|---|---|---|---|
| Nr. of exp. | | mg $CO_2$ prod. in 60 min. per 100 $cm^2$ leaf area | mg $CO_2$ assimil. in 60 min. per 100 $cm^2$ | ratio assim. resp. | decrease in % | |
| | | | | | resp. | assim. |
| 1*b*. | 0Mn | 1,97 | 4,92 | 2,5 | — | — |
| 2*a*. | +Mn | 2,20 | 12,18 | 5,5 | | |
| 2*b*. | 0Mn | 1,79 | 10,91 | 6,1 | 18,6 | 10,4 |
| 3*a*. | +Mn | 2,72 | 7,46 | 2,7 | | |
| 3*b*. | 0Mn | 1,63 | 3,65 | 2,2 | 40 | 51,2 |

It is evident that in all cases respiration calculated per 100 $cm^2$ of leaf area is less in the manganese-deficient plants than in those with sufficient manganese.

However, to some extent this might be a secondary effect due to the fact that by decreased photosynthesis the quantity of assimilates available for respiration is decreased as well. Therefore it is better to compare the decrease in assimilation with the corresponding decrease in respiration; only when the latter exceeds the former notably, a decided influence of manganese on respiration is evident. This is only the case in experiment 2*b*, where the decrease in respiration exceeds the decrease in assimilation by 83%.

This problem is rather a complicated one and it will be necessary to investigate this point more systematically.

The *necrotic spots*, which appear on the leaves of manganese-deficient oatplants are of dual origin, as has been shown previously by the author [8]). Typical brown or yellow spots are caused by ascending oxic metabolic substances produced by bacteria, which attackt the root tips of plants, poor in manganese; this can be

prevented by sterilizing the soil with formalin (not with heat, as this increases the amount of available manganese), whereas they reappear, when the culture medium is reinfected with the original soil.

Necrotic spots of a totally different origin appear on leaves of plants, in a more advanced stage of the disease, when the manganese content is very low. In such cases a serious chlorosis finally develops and then necrosis of rows of cells situated between the green vascular bundles soon extends towards the top and the base of the leaf; this necrosis is a direct consequence of reduced photosynthesis, which is unable to make up for the loss of assimilates by respiration and so inevitably leads to the death of the cells.

The interesting observation of Leeper [11]) that every oatplant investigated as well as the leaves of Canary grass (*Phalaris minor*), which showed marked symptoms of manganese deficiency, gave an intense reaction for nitrate, fits well into the scheme, as it is obvious that accumulation of inorganic nitrogen must take place in leaves, in which insufficient carbohydrates are synthesized to combine with the nitrogen to build up proteins.

The question arises whether the accumulation of nitrate in the leaves is not caused by the accumulation of nitrate in the roots since it has been shown by Burström [2]) that manganese catalyses nitrate assimilation and therefore nitrate might accumulate in the absence of manganese.

Experiments by Mulder *), however, failed to show any difference between the yields of oats grown with nitrate or with ammonia nitrogen and with increasing quantities of manganese in the culture solution. Similar results were obtained with *Aspergillus niger* as a test organism. In this experiment ammonium nitrogen gave considerably higher yields than nitrate when the iron or molybdenum supply was inadequate, indicating the importance of iron and molybdenum for nitrate assimilation.

As Burström uses the roots of four days old oat seedlings, which always contain appreciable amounts of manganese, and especially in his experiments with macerized roots the quantity of manganese to produce this effect was rather high, it is questionable whether his results may be applied to nitrate assimilation

*) The author is very much indebted to Dr. Mulder for the personal communication of his unpublished results.

in general. It is the author's opinion that in this case the reduced production of assimilates gives a satisfactory explanation of the presence of unusually large amounts of nitrates in the leaves.

Finally there is another interesting difference between the leaves of healthy and manganese-deficient plants which has been shown by the author (l.c. p. 223). When put into a 0,1% aqueous solution of phenolred, the leaves of diseased plants lose their turgor even within a few hours, they become slack and shrivel within 24 hours. After 48 hours they are dead and dried up (see photograph fig. 5). Even apparently perfectly healthy leaves of plants poor in manganese behave in this way whereas the leaves of normal healthy plants remain fresh and vigorous for 3–5 days, although the vascular system is packed with phenolred.

According to Danielli[3]) the failure of a dye to enter a plant cell may be due to inability to penetrate the cellulose surrounding them. If this is true, it offers a plausible explanation in the case of manganese deficient plants, where the cellwalls often remain thin due to lack of assimilates and so the dye enters these plant cells more easily. Moreover the protoplast will be thinner and according to Went (personal communication) the permeability will increase with reduced $CO_2$ assimilation. As permeability is so closely related to moleculair structure of the membranes, the dependance of the entrance of phenolred into the cells and the poisoning of the protoplast upon the manganese content of the plant, is obvious.

*The roots.* That the roots of plants deficient in manganese are much smaller than those of healthy plants, is to be expected. Moreover the root tips of these plants are very often decayed, having a glassy appearance and may be coloured red when put in phenolred, because of the presence of alkaline metabolic products produced by the invading microorganisms.

Microscopic examination shows that the root tips often are surrounded by countless microorganisms, whereas in those cases, where manganese has been supplied, root tips and root caps are intact (see fig. 6*a* en *b*).

Bacterial counts have shown that the root tips of manganese-deficient plants contain 4–5 times as many bacteria as those of normal plants.

The effect of an insufficient supply of carbohydrates on the

Fig. 6a. Microphotograph of the root tip and root cap with calyptric cells of an 18 days old healthy oatplant from a water culture with $MnSO_4$ 1 part in 5 million. Roots and leaves were healthy. pH 6.0 (150x).

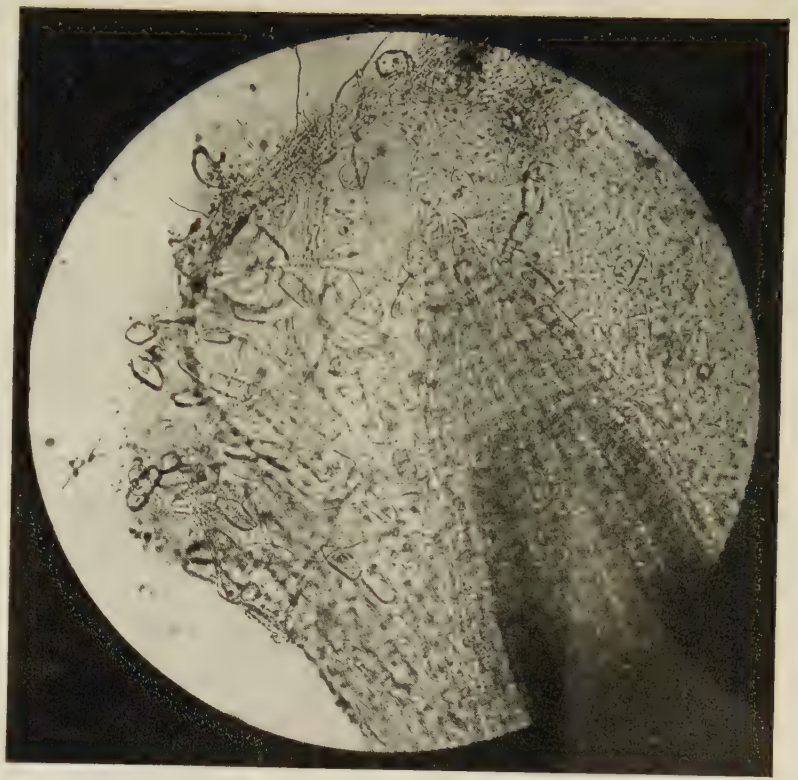

Fig. 6b. Microphotograph of the root tip and root cap of an 18 days old manganese deficient oatplant, from a waterculture without $MnSO_4$. Leaves normal, part of the root tips glassy, and decaying; all root caps perforated by moldmycelia and numerous bacteria (150x).

Fig. 5. Differentiation between apparently healthy leaves of plants very poor in manganese (left), moderate deficient in manganese (middle) and normal (right).
Upper row: leaves, immediately after putting them in 0.1 % phenolred.
Lower row: 24 hours later.

development of the roots becomes manifested in a much slower multiplication of the cells in the meristematic zone, sometimes visible as a stricture; the walls of the cells are thinner and the shedding of the calyptric cells is slowed down. It is clear that the protective function of the rootcap against the entrance of microorganisms into this vulnerable tissue depends upon its rapid rejuvenation, which is impossible, when, by lack of manganese, photosynthetic activits is greatly reduced.

*Acknowledgement.* The author is very much indebted to Dr. J. A. Harley of the Dept. of Botany of the University of Oxford, for his kindness in correcting the manuscript grammatically.

**Summary**

It has been proved that manganese deficiency results in a reduced carbon dioxide assimilation. Different symptoms of manganese deficiency of oats could be traced back to shortage of assimilates in the leaves. This is particularly true for reduced growth, the bending over with a kink of the leaves, the necrotic leaf spots, the withering of the diseased leaves with phenolred, the accumulation of nitrates in the leaves and the reduced resistance of the roots towards bacterial infection.

Received February 11th, 1949.

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Plant and Soil I, no 4 April 1949

# THE MICRO-DETERMINATION OF COBALT IN PLANT MATERIAL

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1. *Introduction.* The diseases of cattle known as "Pining", "Enzootic Marasmus", "Morton Mains disease" and "Bush sickness" have occurred in countries as widely separated as Scotland and Australia and New Zealand. During the last ten years experiments have shown the relationship of these diseases to a deficiency of cobalt (See a.o. reviews of the literature by Russell [1]) and Stiles [2]). In the Netherlands [3]) and also in Denmark attention is being paid to cobalt deficiency as a possible cause of diseases.

In this connection several methods for the micro-determination of cobalt in fodder (grass, hay) and animal tissue (liver) were worked out. The methods previously used for estimation of cobalt in biological materials differed widely and were unsatisfactory in some cases; it was therefore decided to work out a method specially suited to the requirements of the present investigation. The first consideration was accuracy of the method which should also be as simple as possible in view of the large number of samples of material to be analysed.

As in the case of estimation of other inorganic constituents of plant material the method of digestion of the sample is important. Since some authors (Seekles, c.s.) [4]) have referred to losses of cobalt during ashing, digestion has been carried out using sulphuric and nitric acids. It should also be noted that other workers (Ellis and Thompson) [5]) are of the opinion that good results can also be obtained by dry ashing if certain precautions are taken. The possibility of dry ashing and simplification of the wet digestion method are to be studied separately (Hiscox) [6]).

In the colorimetric estimation of cobalt most investigators have

used nitroso-*R*-salt (1-nitroso-2-hydroxy-3.6.-naphthalene disodium sulphate) which forms an intensely red coloured complex with cobalt. Seekles (l.c.) and Claassen and Westerveld[15]) also used this method in recent investigations.

Ellis and Thompson[5]) determined cobalt as a ligroin soluble complex salt of *o*-nitroso-cresol (1-methyl-3-hydroxy-4-nitrosobenzene). This method has a higher sensitivity than the *R*-salt method, but the procedure is more complicated. Using the method of Ellis and Thompson it is possible to determine very small traces of cobalt in milk (0.6–0.7 $\gamma$ per litre). Such sensitivity is not required in the analysis of plant material as the content of cobalt frequently amounts to more than 100 $\gamma$ per kg of dry matter.

2. *The colorimetric estimation of traces of cobalt using nitroso-R-salt. a.* The standard cobalt solution. For the preparation of a calibration curve and routine checking of the photoelectric colorimeter a solution containing 1 $\gamma$ of cobalt per ml. was used. This was obtained by diluting a solution containing 100 mg of cobalt per litre immediately before use. In the preparation of the standard solution 2 g of cobalt carbonate p.a. were dissolved in a small excess of hydrochloric acid after which 1.2 ml of concentrated sulphuric acid were added. After evaporation on a steam bath the excess of sulphuric acid was gradually driven off by heating on a hot air bath and the residue finally heated at 250–300°C to constant weight. Of the dry cobalt sulphate thus obtained 263 mg (100 mg Co) were dissolved in one litre of distilled water containing 1 ml of concentrated sulphuric acid.

It is possible to start from pure cobalt acetate or cobalt nitrate as well as cobalt carbonate in the preparation of the standard cobalt solution.

*b.* Preparation of the calibration curve. At first the method of Bayliss and Pickering[7]) was used for the production of the cobalt nitroso-*R*-salt complex. In this method the excess of *R* salt is removed by boiling with bromine water; this treatment does not discolour the cobalt complex. A good calibration curve was obtained by the use of this method but the procedure is somewhat tedious and does not link up so well with the method ultimately used in the preparation of samples. As in the case of other methods the more laborious one of Bay-

liss and Pickering is also interfered with by the presence of copper and iron in the solution. A modified form of the method used by Kidson and Askew[8]) (see also Seekles c.s.)[4]) proved relatively simple and satisfactory.

The photo electric colorimeter was used with light filters different from those used by previous workers. The filters used in the present work were:

Schott & Gen., Jena, Glass filter BG 12, Maximum transmission 415 $\mu$ blue.

Schott & Gen., Jena, Glass filter BG 7, Maximum transmission 490 $\mu$ blue-green.

Since the experimental solution contains 10–12 drops of concentrated sulphuric acid after preparation from the sample, this quantity of sulphuric acid was always added to the standard solution used in the preparation of the calibration curve.

A mixture of the required quantity of diluted standard cobalt solution, 10–12 drops of concentrated sulphuric acid and 1 ml of water contained in a 100 ml flask, is neutralised with KOH (20%) using 2 drops of phenolphthalein solution. The mixture is then weakly acidified with 0.25 N H Cl and concentrated over a small flame to a volume of about 3 ml. The method used in the development of the Co-*R*-salt complex is dealt with in section 4,*d*. The data obtained in this way for the calibration curve are shown in table I. The extinctions were obtained by the use of cells having a light path of 3 cm.

TABLE I

| Quantity of Co($\gamma$) in the solution | Extinction with filter BG 12 | Extinction with filter BG 7 |
|---|---|---|
| 0 | 0.207; 0.214; 0.207; 0.207 | 0.053; 0.055; 0.054 |
| 1 | 0.247 | |
| 3 | 0.313; 0.326; 0.325; 0.325; 0.318 | 0.130; 0.130; 0.130 |
| 5 | 0.391; 0.393; 0.395 | 0.182; 0.183 |
| 6 | 0.433; 0.432; 0.455 | 0.203; 0.212 |
| 10 | 0.580 | |

The extinction obtained with 0 cobalt due to excess of *R*-salt is higher for filter BG 12 than for filter BG 7. In spite of this filter BG 12 was preferred and was used with the experimental solutions as the extinction for a given amount of cobalt were larger. These findings agree with those of Willard and Kaufman[9]) who found the maximum absorption for the Co-*R*-salt complex to be at

about 415 mμ. The extinction obtained for the blank was much lower when the excess of *R*-salt was destroyed by bromination by the method of Bayliss and Pickering (l.c.).

A linear relationship between extinction and cobalt concentration was obtained for both light filters. On comparing the cobalt solution with the blank the following extinction was obtained with filter BG 12:

$$E\ (1\ \gamma \text{ per } 1 \text{ ml, cell } 1 \text{ cm}) = 0.313$$

The Co-*R*-salt complex is slightly sensitive to light so that whilst the extinction given by a solution standing for 16 hours in the dark was 98% of the initial value, the extinction given after standing for 36 hours on a table (about 24 hours in diffuse daylight) had decreased to 82% of the initial value. It is therefore advisable to carry out the determinations as quickly as possible or at any rate avoid exposure of the solution to daylight.

*c.* The influence of iron and copper. In the development of a method for the determination of cobalt in plant material it was necessary to study the influence of iron and copper on the colorimetric determination. For this purpose varying quantities of iron and copper were added to solutions containing 4 $\gamma$ of cobalt and their effect on the results observed. The method of preparation of the cobalt-*R*-salt complex was the same as that used for preparing the calibration curves. The iron was added as ferric salt. The results set out in the following table show the values obtained after correction for the cobalt present in the iron-salt (0.15$\gamma$ per mg of Fe).

TABLE II

| 4$\gamma$ Co | | $\gamma$ Co, found according to the calibration curve | |
|---|---|---|---|
| $\gamma$ $Fe^{+++}$ | $\gamma$ $Cu^{++}$ | Filter BG12 | Filter BG7 |
| 0 | 0 | 4.1; 3.9 | 4.0; 4.0 |
| 100 | 0 | 4.0 | 3.9 |
| 500 | 0 | 3.1; 3.6; 3.4; 3.7 | 2.8; 3.6; 3.2; 3.7 |
| 2500 | 0 | 3.8 | 3.5 |
| 5000 | 0 | 4.8; 3.4 | 4.1; 3.0 |
| 10000 | 0 | 4.7 | 4.0 |
| 0 | 150 | 4.0; 4.1; 4.0; 4.1 | 4.0; 4.2; 4.0 ;4.2 |
| 0 | 300 | 3.9; 3.8 | 3.9; 3.8 |
| 0 | 400 | 3.6 | 3.6 |
| 0 | 500 | 3.4; 3.5; | 3.4; 3.6 |
| 100 | 150 | 4.0; 4.0 | 4.1; 3.9 |
| 100 | 250 | 4.1; 3.9 | 4.2; 3.9 |
| 100 | 300 | 3.8; 3.9 | 3.9; 3.8 |

The results in Table II indicate that in these experiments the interference by iron was very irregular in magnitude and no decisive explanation for this could be found. It is possible that the *R*-salt is partially oxidised by the ferric-iron before the Co-complex is formed. In the presence of large amounts of iron the colour of the solution turned red or green after addition of the *R*-salt. It may be assumed that small amounts of iron (less than 100 $\gamma$), either when alone or in combination with copper (less than 250 $\gamma$), will not interfere in the determination of traces of cobalt. Larger amounts of copper (more than 250 $\gamma$) may give rise to values which are too low.

The amount of iron in plant materials is very variable, ranging from 10 to 1000 mg per kg of dry substance. This means that if in the determination of cobalt 10 gr of dry material are used there will be 100–10,000 $\gamma$ of iron in the final solution. It is therefore advisable to remove the iron before development of the Co-*R*-salt complex. A residual amount of copper up to 250 $\gamma$ after the removal of the iron will not affect the results. When analysing 10 g of dry material the copper content must not be more than 25 mg per kg, and if the determination is carried out on 5 mg of dry material 50 mg of Cu per kg of dry substance can be tolerated. It can therefore be deduced that it is not necessary to remove copper before the determination of cobalt in pasture samples.

Marston and Dewey[10]) developed a method for the determination of cobalt after removal of iron and copper; the method, however, is very tedious. Interference by iron, when present in large quantities, has been noted by previous workers (McNaught[11]), Ellis and Thompson[5])). On the other hand, other workers are of the opinion that it is not necessary to remove iron (Kidson and Askew[8]), Seekles c.s.[4])). In a recent investigation Claassen and Westerveld[15]) found that even 125 mg of ferric iron did not interfere with the cobalt estimation. It must be remembered that the results shown in table II may only be valid in conjunction with the light filters specified and it may well be that different results will be obtained when other filters are used.

3. *The separation of iron and cobalt.* In order to overcome possible interference by iron an attempt was made to remove iron from the

digested material by extraction with ether according to the method used by McNaught [12]). This method did not prove successful as there was partial extraction of the cobalt and consequently results which were too low were obtained.

As it was evident that extraction of iron from the digested material was attended by difficulties it was decided to try and extract the cobalt. This method also has the advantage that other interfering salts are eliminated.

Extraction of cobalt and copper as thiocyanates in a mixture of amylalcohol and ether gave results which were too low.

According to Ellis and Thompson [5]), sodium diethyldithiocarbamate in solution with ammonium citrate at pH 6,5, forms complex compounds with Co and Cu which are soluble in carbon tetrachloride. This method was used and after distilling off the solvent from the combined carbon tetrachloride extracts it was found that the residue could be digested easily with concentrated sulphuric and nitric acids. The determination of cobalt was then carried out according to the procedure described in section 4,*d*. With solutions containing only cobalt salts this method gave excellent results but in the case of digests of plant material, extraction of cobalt by this method proved to be incomplete.

Good results were obtained by extraction of cobalt (and copper) from solutions containing sodium citrate with dithizone at pH 8.5. This method was also recommended by Ellis and Thompson. Details of the method are given in the next section.

4. *The analytical procedure for the determination of cobalt in plant-materials.* Outline of procedure. *a*. 5 or 10 g of the dried and ground sample are wet ashed with nitric and sulphuric acids.

*b*. After neutralisation with ammonia the cobalt is extracted at pH 8.5 by means of a solution of dithizone in carbon tetrachloride the formation of a precipitate being prevented by the addition of sodium citrate.

*c*. The dithizone extract is digested with nitric and sulphuric acids.

*d*. The cobalt in the digest is determined by the use of nitroso-*R*-salt according to the modified procedure of Kidson and Askew. The complexes formed by metals other than cobalt and nitroso-*R*-salt are destroyed by boiling with nitric acid. Finally eth

concentration of the solution is determined by means of a photo-electric colorimeter using a 3 cm cell.

Reagents required:

1. Nitric acid (65%)
2. Sulphuric acid (concentrated)
3. Sodium citrate solution (20%)
4. Ammonia (25%)
5. Phenolphthalein (0.1% solution in 95% ethanol)
6. Dithizone (0.2% filtered solution in pure carbon tetrachloride)
7. Potassium hydroxide solution (20%)
8. Hydrochloric acid (0.25 Normal solution)
9. Nitroso-*R*-salt solution (0.2% in water)
10. Sodium acetate solution (100 g in 250 ml).

All the reagents should be as chemically pure as possible. The water, nitric acid and ammonia are distilled from all glass Pyrex stills. The carbon tetrachloride is repeatedly shaken with concentrated sulphuric acid, washed with water, dried over solid sodium hydroxide and finally distilled. The solutions of dithizone and nitroso-*R*-salt are stored in a cool dark place.

All glassware is cleaned with warm nitric acid and water.

*a.* Digestion. 5 g of the sample, 10 ml of distilled water, 10 ml of 65% nitric acid and 5 ml of concentrated sulphuric acid are carefully mixed in a 250 ml flask *). The mixture is gently heated until it begins to carbonise, then a few drops of 65% nitric acid are added and the digestion slowly continued until the solution turns black. Further additions of nitric acid are made until the solution becomes colourless, and remains completely colourless on cooling; 20 ml of water are then added and the solution heated on a steam bath for one four to destroy nitroxylsylphuric acid.

*b.* Extraction of cobalt with dithizone. A further 20 ml of water are added, and after boiling for a short time the solution is transferred to a 250 ml separating funnel, the flask being rinsed a few times with hot water acidified with sulphuric acid. After adding 15 ml of the 20% sodium citrate solution the separating funnel and contents are cooled under the tap; 40 ml

*) With 10 g of sample, 20 ml of water, 15 ml nitric acid and 6 ml of sulphuric acid are used.

of a 0.2% solution of dithizone in carbon tetrachloride is then added along with 10 drops of phenolphthalein.

Concentrated ammonia is added drop by drop whilst the mixture is being shaken. Before the phenolphthalein takes on a permanent red colour the solution is again cooled and then ammonia carefully added until the aqueous layer becomes distinctly red. The mixture is now vigorously shaken for one minute. Occasionally at this stage the mixture becomes turbid but this does not interfere with subsequent procedure. The dithizone extract is run off into a 100 ml flask and the remaining aqueous layer shaken with a further 10 ml of the dithizone solution. The extraction is repeated until the dithizone solution remains green. Finally the aqueous layer is washed twice with 10 ml of carbon tetrachloride. The carbon tetrachloride is distilled off on a steam bath from the combined dithizone extracts and the bronze coloured residue digested in the same flask.

*c.* Digestion of the residue from the dithizone extract. To the residue are added 10–12 drops of concentrated sulphuric acid and 25 drops of nitric acid the mixture being heated gently over a small flame, the flask being placed in an inclined position on a copper gauze. If necessary further drops of nitric acid should be added after cooling and the digestion continued until the solution becomes almost colourless. Heating is then continued for a further half hour. After cooling the digest 5 ml of water are added and the mixture heated, to destroy nitroxylsulphuric acid, on a copper gauze until $SO_3$ fumes are given off. 1 ml of water and two drops of phenophthalein are then added and the solution made just alkaline with 20% KOH. This solution is acidified with an excess of about 10 drops of 0.25 N hydrochloric acid, then concentrated over a small flame to a volume of about 3 ml. During subsequent cooling colourless salts crystallise but these do not interfere with further procedure.

*d.* Development of the Co-nitroso-*R* salt complex. To the solution of dithizone extract digest, 0.5 ml of 0.2% nitroso-*R*-salt solution, 2 ml of 40% sodium acetate and 20% potassium hydroxide are added in succession until the phenophthalein turns red. 0.25 N hydrochloric acid is then immediately added until the pink colour has just disappeared; precise adjustment of the reaction is important. The mixture is boiled for exactly one minute (the flask being in an inclined position) and after adding 2 ml of

concentrated nitric acid is boiled again for one minute. The solution is then transferred to a 25 ml volumetric flask and after cooling in a dark place made up to 25 ml, mixed and readings taken on the photo electric colorimeter using a 3 cm cell (See also section 2,*b*).

The cobalt content of the reagents used was ascertained by means of blanks and the colorimeter readings differed only slightly from the value for 0 γ Co on the calibration curve (see section 2,*b*). The reagents used are therefore almost free from cobalt.

5. *Accuracy of the method.* The accuracy of the method was ascertained firstly by determining the recovery rate for cobalt added before wet ashing and secondly by comparison of the results of estimations on 5 g and 10 g of the same sample. The results of these tests of accuracy are given in Table III and were carried out on 14

TABLE III

| Sample No. | Weight of sample (grams) | Added cobalt (γ) | Cobalt content (γ) | Recovery of added cobalt (γ) | Cobalt content of sample (mg/kg) |
|---|---|---|---|---|---|
| 1*a* | 10 | 2 | 5.13 | 1.87 | 0.31 |
| *b* | 10 | — | 3.26 | | 0.33 |
| 2*a* | 10 | 2 | 5.00 | 2.03 | 0.30 |
| *b* | 10 | — | 2.97 | | 0.30 |
| 3*a* | 10 | 2 | 3.49 | 1.89 | 0.15 |
| *b* | 10 | — | 1.60 | | 0.16 |
| 4*a* | 10 | 2 | 3.65 | 1.95 | 0.17 |
| *b* | 10 | — | 1.70 | | 0.17 |
| 5*a* | 10 | 2 | 3.45 | 1.85 | 0.15 |
| *b* | 10 | — | 1.60 | | 0.16 |
| 6*a* | 10 | 2 | 5.34 | 1.97 | 0.33 |
| *b* | 10 | — | 3.37 | | 0.34 |
| 7*a* | 10 | 2 | 3.63 | 2.00 | 0.16 |
| *b* | 10 | — | 1.63 | | 0.16 |
| 8*a* | 10 | 2 | 4.35 | 1.95 | 0.24 |
| *b* | 10 | — | 2.40 | | 0.24 |
| 9*a* | 10 | 2 | 8.04 | 2.07 | 0.60 |
| *b* | 10 | — | 5.97 | | 0.60 |
| 10*a* | 5 | 2 | 2.88 | 2.00 | 0.18 |
| *b* | 5 | — | 0.88 | | 0.18 |
| 11*a* | 5 | 2 | 3.10 | 2.05 | 0.22 |
| *b* | 5 | — | 1.05 | | 0.21 |
| 12*a* | 10 | — | 3.25 | | 0.33 |
| *b* | 5 | — | 1.70 | | 0.34 |
| 13*a* | 10 | 2 | 3.25 | 1.95 | 0.13 |
| *b* | 5 | — | 0.65 | | 0.13 |
| 14*a* | 10 | 2 | 8.38 | 2.22 | 0.64 |
| *b* | 5 | — | 3.08 | | 0.62 |

samples of dried fresh grass. Recovery of added cobalt (2 $\gamma$) ranged from 1.9 $\gamma$ to 2.2 $\gamma$ i.e. 95–110%. The average recovery rate was 99% i.e. 1.98 $\gamma$ which may be regarded as satisfactory. Analysis of 5 g and 10 g lots of the same sample (experiment No. 12, 13 & 14) gave no significant discrepancies.

Tests were also made to ascertain whether or not the cobalt was extracted quantitatively by the dithizone from the solutions. During a routine analysis 3.0 $\gamma$ of cobalt were added to the aqueous phase from which cobalt had already been extracted with dithizone. This aqueous phase was then subjected to normal procedure (extraction with dithizone, treatment with *R*-salt and colorimeter reading) when 3.0 $\gamma$ of cobalt were recovered.

Finally the following tests were carried out with one sample of dried grass.

*a*. The unmodified procedure as described in Section 4 was carried out.

*b*. After digestion of the plant material the solution was filtered before extracting with dithizone the filter and digestion flask being washed with weakly acidified water. This procedure may be necessary when the sample contains insoluble material such as soil.

*c*. After heating the digest with water and evaporating, the calcium salts were precipitated by addition of 20 ml N sulphuric acid and 25 ml of ethanol. The precipitate was filtered off and the precipitate and filter washed with the same sulphuric acid-ethanol mixture. The filtrate and washings were evaporated and the sulphuric acid driven off. The residue was then dissolved in 15 ml of 20% sodium citrate and hot water, and the resulting solution transferred to a separating funnel. The normal procedure was then followed. The results of these three tests are summarised in Table IV.

TABLE IV

| Procedure | Weight of sample (grams) | Cobalt content mg/kg |
|---|---|---|
| *a* | 10 | 0.28 |
| *b* | 10 | 0.30; 0.29 |
| *c* | 10 | 0.30 |

The amount of iron present in the dithizone extract is always very small, in any case less than 100 $\gamma$ so that subsequent interference by iron is excluded.

6. *Combined determination of cobalt and copper.* After determination of the cobalt, 15 ml of the 25 ml of Co-*R*-salt solution were evaporated to dryness and the residue digested with 30 drops of concentrated sulphuric acid and 10 drops of nitric acid. Digestion with 1 ml of 30% $H_2O_2$ is repeated three times and then 10 ml of water and 10 ml of 20% sodium citrate added. The copper content is then determined colorimetrically with diethyl dithiocarbamate as described in a previous paper [14]). When 5 g of the sample have been treated in this way the amount of copper is determined in $\frac{15}{25} \times 5 = 3$ g of material. As in the case of cobalt, the copper content of the reagents is ascertained on a blank determination.

Determination of copper content was carried out on a small number of samples and the results are given in Table V; column *a* shows the copper contents as determined by the normal method (14) and column *b* shows values obtained when cobalt and copper are determined in the same aliquot of material.

TABLE V

| Sample no. | Cobalt content mg/kg | Copper content (mg/kg) | |
|---|---|---|---|
| | | *a* | *b* |
| 15 | 0.21 | 6.5 | 7.0 |
| 16 | 0.32 | 9.3 | 8.5 |
| 17 | 0.17 | 8.5 | 8.8 |
| 18 | 0.12 | 10.0 | 10.4 |
| 19 | 0.21 | 10.4 | 10.6 |
| 20 | 0.32 | 13.9 | 13.7 |
| 21 | 0.36 | 14.3 | 14.7 |

7. *Results of the determination of cobalt in samples of grass and hay.* The cobalt content of 70 samples of dried grass and hay was determined according to the procedure described above. The agricultural significance of these results will not be discussed here. Table VI shows the results arranged in the form of a frequency table.

8. *Contamination of samples with soil.* The high values for cobalt content occurring in Table VI will for the most part be caused by the presence of soil in the sample. The utmost precaution must be taken to avoid contamination of samples with soil. According to P i p e r [13]) cobalt occurs in soils in concentrations 20–100 times as great as in plants. Samples of sand and clay were analysed from

TABLE VI

| Cobalt content (mg/kg of dry substance) | Number of samples |
|---|---|
| 0.06–0.10 | 10 |
| 0.11–0.15 | 14 |
| 0.16–0.20 | 15 |
| 0.21–0.25 | 16 |
| 0.26–0.30 | 6 |
| 0.31–0.35 | 3 |
| 0.51–0.65 | 5 |
| 1.66–1.70 | 1 |
| | Total 70 |

the upper part of the sod layer (0–5 cm). The cobalt was determined in 0.5–1 g of the samples of dry soil according to the procedure described above including digestion with sulphuric and nitric acids. The results obtained were as follows:

| | Co (mg/kg) |
|---|---|
| sand | 2.1 |
| ,, | 3.9 |
| clay | 9.0 |
| ,, | 12.9 |
| ,, | 10.4 |
| ,, | 6.2 |

A simple example will clearly illustrate the influence of contamination with soil on the analytical results.

If the cobalt content of grass not contaminated with soil is 0.1 mg per kg dry substance and if then only 1% of the dry matter is replaced by clay of cobalt content 10 mg per kg the analysis will show a cobalt content of the contaminated grass of 0.2 mg per kg.

Similarly a 5% contamination of the dry material with clay would give an apparent cobalt content of 0.6 mg per kg!

**Summary**

The determination of cobalt in plant material has been investigated. The method adopted is based on the colorimetric determination of the cobalt-nitroso-*R*-salt complex. Interference by iron is avoided by extraction of the cobalt from the digestion solution by means of dithizone. The method has several advantages. The tedious fuming off of sulphuric acid and removal of calcium salts is unnecessary, and the determinations of cobalt and copper in the same aliquot are possible.

Figures are given for the cobalt content of fresh grass and hay. Much too high results will be obtained if the samples are contaminated with soil.

Received January 12th, 1949.

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